DEGRADATION AND BIOLOGICAL ACTIVITY OF ALPHA-TOCOPHEROL DURING STORAGE IN A DEHYDRATED MODEL FOOD SYSTEM

BY

WARREN A. WIDICUS

A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL
FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1980

To My Wife, Mary

ACKNOWLEDGEMENTS

The author wishes to express sincere thanks to Dr. J. R. Kirk, his major professor, for support and invaluable guidance during this study and the writing of this dissertation. Special gratitude is also extended to Dr. J. F. Gregory III for continual encouragement and counsel throughout the work and to Dr. J. A. Cornell for helpful advice and assistance concerning the statistical evaluation of the experimental results.

The author expresses grateful appreciation to the members of his graduate committee, Dr. L. H. Bailey, Dr. J. A. Cornell, Dr. J. F. Gregory III, and Dr. H. A. Moye for serving on the committee and for reviewing this manuscript.

Appreciation is extended to the Department of Food Science and Human Nutrition, University of Florida, for facilities and financial support during this research. The author also acknowledges financial support for this research from the General Foods Corporation.

Finally, the author extends most sincere thanks to his wife, Mary, for constant inspiration and encouragement throughout his graduate studies.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF TABLES

LIST OF TABLES (Continued)

LIST OF FIGURES

LIST OF FIGURES (Continued)

Abstract of Dissertation Presented to the Graduate Council of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

DEGRADATION AND BIOLOGICAL ACTIVITY OF ALPHA-TOCOPHEROL DURING STORAGE IN A DEHYDRATED MODEL FOOD SYSTEM

By

Werren A. Widicus

December, 1980

Chairman: James R. Kirk
Major Department: Food Science and Human Nutrition

All animals, including humans, require a dietary source of vitamin E. Cereals and grains are a major source of vitamin E for the world's population. During processing and storage, the vitamin E content of foods is known to decrease. Additional research characterizing the factors that cause destruction of vitamin E is needed. There have been only a few reports that characterize the biological activity of vitamin E in stored foods. This study was designed to characterize the parameters which influence the storage stability and biological activity of α-tocopherol, the most potent and predominant form of vitamin E.

The storage stability of α-tocopherol in a dehydrated model food system was studied. The storage parameters which were varied included: lipid composition (no lipid, saturated lipid, or unsaturated lipid), the water activity (a_w) (0.10, 0.24, 0.42, and 0.83); the storage temperature (20, 30, and 37°C), and storage container oxygen content (0.05 or 4.8 mmol gaseous oxygen per container).

Alpha-tocopherol degraded in a first order fashion when stored in the dehydrated model food system containing no lipid or a saturated lipid (hydrogenated coconut fat). The rate of α-tocopherol degradation decreased as a function of a_w through the range of 0.65 to 0.10. The rate of α-tocopherol degradation was higher ($p \leq 0.05$) when the oxygen content in the storage container was increased from 0.05 to 4.8 mmol oxygen. This relationship was most prominent at higher a_ws and storage temperatures and was attributed to the availability of oxygen at the interface between α-tocopherol and water. The thermodynamic activation parameters including the apparent activation energies and the Gibbs free energy of activation were not effected significantly ($p \leq 0.01$) by a change in the a_w or oxygen content in the storage container, indicating that the mechanism of α-tocopherol degradation was not altered by changes in the storage conditions used in this study.

During storage of the model food system containing α-tocopherol and methyl linoleate, losses of either α-tocopherol or methyl linoleate could be modeled by zero order kinetics. The loss of α-tocopherol did not follow simple zero order kinetic theory because the degradation rate of α-tocopherol and methyl linoleate were effected by the initial α-tocopherol concentration. A complex reaction involving competition of α-tocopherol and methyl linoleate was proposed.

The rate of α-tocopherol loss in a dehydrated model food system containing methyl linoleate was influenced by the a_w of the model system, the storage container oxygen content, and the storage temperature. The rate of α-tocopherol loss was the slowest at 0.23 a_w, a value near the B.E.T. monomolecular moisture content, and increased as the a_w was decreased below the B.E.T. monomolecular moisture content (to 0.11) or

as the a_w was increased in the multilayer region (to 0.42). The rate of α-tocopherol loss decreased as the a_w (0.67) approached the region of capillary hydration. Alpha-tocopherol degraded more rapidly ($p \leq 0.01$) in the model food system stored with limited headspace due to the different composition and/or concentration of reactants formed during storage with limited oxygen. Alpha-tocopherol degraded almost completely when stored with 4.8 cmol oxygen, but degraded only to approximately 20 μg/g and remained constant when stored in a container with 0.05 cmol oxygen. The apparent activation energies were not significantly affected ($p \leq 0.01$) by the initial α-tocopherol concentration, storage container oxygen content, or a_w, indicating that the mechanism of α-tocopherol degradation was not altered by these storage parameters.

The biological activity of α-tocopherol remaining in the model system after storage was evaluated by a rat bioassay. In addition to the stored model system, synthesized degradation products of α-tocopherol were evaluated for their vitamin E biological activity.

The α-tocopherol remaining after storage of the model systems containing no fat or saturated fat was biologically active. Following storage, the biological activity of α-tocopherol in the model system containing methyl linolenate was lower, indicating that α-tocopherol degradation may occur during digestion and absorption in the rat. Two degradation products of α-tocopherol, α-tocopheryl oxide and α-tocopheryl quinone, exhibited vitamin E biological activity.

The data from these experiments indicate that the a_w, storage container oxygen content, temperature, and lipid composition affect the degradation rate and pattern of α-tocopherol degradation. These factors must be controlled to minimize the loss of α-tocopherol during storage of foods.

INTRODUCTION

Although all animals, including humans, require a dietary source of vitamin E, the dietary requirements for vitamin E have not been well established. Two U.S. dietary surveys by Bunnell et al. (1965) and Sisri and Evarts (1973) reported average human daily intakes to be 7.4 mg or 9.0 mg of α-tocopherol, respectively. The National Academy of Sciences' Food and Nutrition Board (1980) reported that a balanced diet should provide an intake of vitamin E large enough to prevent vitamin E deficiency and established a recommended daily allowance of 8-10 mg of α-tocopherol for adults. Cereals and grains are a major source of vitamin E for the world's population.

The vitamin E content of foods is known to decrease during storage. The factors which effect vitamin E stability during storage of dehydrated food products have not been characterized. Specifically, the effects of water activity, storage temperature, oxygen exposure, and lipid composition on the stability of vitamin E have not been evaluated. Labuza (1972) reported that few data are available to predict the storage stability of α-tocopherol. Likewise, the biological activity of α-tocopherol and its decomposition products as a function of processing and storage has not been evaluated.

This study was designed to evaluate the storage stability of α-tocopherol in a dehydrated model food system. A factorial experimental design was used to study the effects of water activity, storage temperature, oxygen availability, and lipid composition. The biological activity of

α-tocopherol and its degradation products were determined following storage of the model food system. The results from this experiment should serve two purposes.

A. Identify the storage parameters which are important in stabilizing α-tocopherol during storage of dehydrated foods and feeds;

B. Provide information characterizing the biological activity of α-tocopherol and α-tocopherol degradation products after storage.

LITERATURE REVIEW

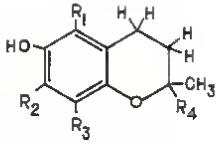
Vitamin E--History

Evans and Bishop (1922) reported the occurrence of a lipid soluble compound which was required to prevent fetal resorption in rats consuming rancid lard. Mattill et al. (1924) later confirmed that a lipid soluble fraction isolated from wheat germ oil prevented rat fetal resorption. Sure (1924) designated this fraction as vitamin E, while Evans and Bishop (1922) referred to the lipid soluble compound as tocopherol [from the Greek words 'tocos' (childbirth), 'pherein' (to bring forth), and 'ol' (alcohol)]. Evans et al. (1936) first reported the isolation of pure vitamin E from the unsaponifiable fraction of wheat germ oil. Fernholz (1938) and Karrer and Fritzsche (1938) were first to report the structure of vitamin E.

Vitamin E--Chemistry

Vitamin E denotes a group of eight tocol [2-methyl-2-(4',8',12'-trimethyltridecyl)-6-chromanol] derivatives, methyl-substituted on the benzene ring, which exhibit vitamin E activity (Karrer and Fritzsche, 1938). The structures of the various forms of vitamin E are shown in Figure 1.

All tocopherols and tocotrienols are lipid soluble and are readily soluble in ethanol, ethyl ether, acetone, chloroform, and benzene. The tocopherols are liquid at room temperature with melting points ranging from -4 to 3.5°C (Robeson, 1943 and McHale et al., 1958). The various forms of vitamin E show UV absorption with λ_{max} ranging from 292 to 298



tocopherole R_4 - CH_2 (CH_2 - CH_2 - CH(-CH_3) - CH_2)$_3$ H

tocotrienol R_4 - (CH_2 - CH_2 - CH = C(-CH_3))$_3$ - CH_3

	R_1	R_2	R_3
α-tocopherol	CH_3	CH_3	CH_3
β-tocopherol	CH_3	H	CH_3
γ-tocopherol	H	CH_3	CH_3
δ-tocopherol	H	H	CH_3
[illegible]-tocotrienol	CH_3	CH_3	CH_3
ε-tocotrienol	CH_3	H	CH_3
η-tocotrienol	H	CH_3	CH_3
δ-methyl-tocotrienol	H	H	CH_3

Figure 1. Structures of the eight vitamin E compounds

ns and $E^{1\%}_{1cm}$ ranging from 75.8 to 103 in ethanol (Kofler et al., 1972). Kofler et al. (1972) also summarized the infrared (IR) spectra, the nuclear magnetic resonance (nmr) spectra, and the optical activity of the eight forms of vitamin E.

Vitamin E--Dietary Sources

Vitamin E is synthesized in plants with the various tocopherols and tocotrienols formed through hydrogenation of intermediate trisnel compounds. Green (1958) reported that α-tocopherol is the predominant form of vitamin E in growing plants, but other tocopherols predominate in seeds. He also reported that the α-tocopherol concentration of germinating seeds increases with time, which leads to an increased ratio of α-tocopherol to other tocopherols in growing plants. α-Tocopherol may be produced by methylation of mono- and dimethylated tocopherols so that generally immature plants contain a higher ratio of β-, γ- and δ-tocopherol to α-tocopherol than do mature plants (Baumgartner, 1977).

Vitamin E is not synthesized by animals, so their needs must be fulfilled by dietary sources. Many review articles summarize present knowledge of the vitamin E content of foods (Bunnell et al., 1965; Ames, 1972; Baumgartner, 1977, and McLaughlin and Weihrauch, 1979). The major natural sources of dietary vitamin E are fats and oils and cereal grains. Other foods may contain vitamin E, but not in sufficient quantity to be considered major dietary sources.

Fats and oils freshly extracted from seeds generally are the richest dietary sources of vitamin E. The total vitamin E concentration and the ratio of the various tocols present depends on: (1) the type of fat or oil (Lease, 1950), (2) the maturity of the seed (Booth, 1964), (3) the genetic variety (Cahell and Ellis, 1942), (4) the length of storage of

the oil (Rammoujan and Anantakrishnan, 1958), and (5) the method of extraction (Gutfinger and Letan, 1974).

Cereal grains are an important dietary source of vitamin E. Grams et al. (1970) reported the vitamin E distribution in corn as follows: germ (70-86%), endosperm (11-27%), and pericarp (2-3%). These data indicate that whole grain products would contain more vitamin E than products produced from the separated endosperm. Bleaching of flour by addition of chlorine dioxide or by aging may cause destruction of as much as 65% of the naturally occurring vitamin E in flours (Fraser and Linen, 1967 and Moore et al., 1957).

Vitamin E--Biological Functions

Evans and Bishop (1922) reported that a lipid soluble nutritional factor was required to prevent fetal resorption in rats. This has caused researchers to study the nutritional action of vitamin E. Green and Bunyan (1969) discussed in detail the current theories concerning vitamin E biochemical activities and classified them into two concepts: (A) Vitamin E is a biological antioxidant, and (B) The metabolic role of vitamin E. The following is a brief summary of these two concepts.

Although Olcott and Emerson (1937) reported that vitamin E exhibited antioxidant activity during the storage of oils, the biological antioxidant theory of vitamin E was first proposed by Davies and Moore (1941). The latter authors suggested that α-tocopherol acted as an in vivo antioxidant for unsaturated lipids in cell membranes and was required to maintain cell integrity. Mattill (1927) was the first to demonstrate the relationship between dietary lipids and vitamin E requirements. He showed that as the concentration of oxidizable lipids in the diet of rats increased, the rate of sterility of the rats increased. Recently, Litov

et al. (1978) reported that during vitamin E deficiency, rats respire increased amounts of pentane, a product of *in vivo* lipid oxidation. This demonstrates a direct relationship between *in vivo* lipid peroxidation and vitamin E deficiency. Although the actual antioxidant mechanism involving α-tocopherol is not known, it is generally accepted that α-tocopherol is required to prevent tissue membrane destruction.

The second basic theory concerns a defined catabolic role for vitamin E. Cassi et al. (1972) reported that the bone marrow enzyme, δ-aminolevulinic acid synthetase, and the liver enzyme, δ-aminolevulinic acid dehydratase, both exhibited decreased activity in vitamin E deficient rats. Bipleek (1974) reported that even though δ-aminolevulinic acid synthetase and dehydrase activities were reduced in vitamin E deficiencies, no overall reduction of heme synthesis was noted. Loodon et al. (1972) reported that the activity of the P-450-dependent microsomal nitroreductase enzyme in the rat was dependent on the vitamin E status. Catignani et al. (1974) reported that rabbit liver xanthine oxidase concentration increased as a function of vitamin E depletion. The results of the above cited studies suggest that vitamin E status affects the activity of many mammalian enzymes. However, studies have not confirmed vitamin E to be a metabolic factor for specific physiological functions.

Vitamin E--Dietary Requirements

Nutritional deficiency diseases associated with low vitamin E intake in humans are not well defined (Binder and Spire, 1967). Human deficiency symptoms developed only after long periods of impaired fat absorption which induced deficiencies of other fat soluble vitamins and essential fatty acids. The National Academy of Sciences' Food and Nutrition Board (1980) reported that there is no clinical or biochemical evidence

indicating that a person consuming a balanced diet will consume an inadequate quantity of vitamin E.

The vitamin E content of diets consumed in the United States was reported by Bunnell et al. (1965) and Hisri and Evarts (1973). Bunnell et al. reported an average daily intake of 7.4 mg α-tocopherol while Hisri and Evarts reported that the typical American diet contains an average of 9.0 mg α-tocopherol per day. As a result of these studies, the 1980 Recommended Dietary Allowance for vitamin E was established at 10 IU for adult males and 8 IU for adult females (National Academy of Sciences, 1980).

Vitamin E--Units and Activity

One International Unit (IU) of vitamin E is defined as the activity exhibited by 1 mg of dl-α-tocopheryl acetate. The activity of synthetic dl-α-tocopherol is 1.1 IU/mg, while that of the naturally occurring d-α-tocopherol is 1.49 IU/mg. Other forms of vitamin E are reported to have lower activities than α-tocopherol. Century and Horwitt (1965) reported the biopotencies of tocols in relation to the biopotency of α-tocopherol as: β-tocopherol (0.37), γ-tocopherol (0.07), and δ-tocopherol (0.22). Sunyam et al. (1961) reported that the tocotrienols all have less than 10% of the activity of α-tocopherol. The National Academy of Sciences recommendation (1980) assumes that 80% of the tocols in the U.S. diet are α-tocopherol, and all recommendations are based on this estimate.

Vitamin E--Methods of Determination

Many chemical methods have been used to determine the vitamin E content of foods. There are eight forms of vitamin E and methods must be developed that quantitate all eight forms, either individually or collectively. Presently, only a few methods are available that are sufficiently

sensitive, accurate, and specific to determine the concentration of a specific form of vitamin E in foods. Major emphasis has been placed on the development of an analytical procedure for the determination of α-tocopherol, the most potent and widely occurring form of vitamin E.

Colorimetric determination of tocols has been accomplished by complexing tocols or tocol-reduced $FeCl_3$ with 2,2'-dipyridyl, 1,10-phenanthroline, 2,2',2"-terpyridine, tripyridyltriazine, or diphenyl (bathe-) phenanthroline (Taco, 1961). These colorimetric procedures are neither specific or sufficiently accurate to use in analysis of naturally occurring tocols. Sheikh et al. (1977) listed the following analytical problems associated with one of the previously listed colorimetric methods:

A. Since the eight tocols have different structures, each tocol forms a different complex with the colorimetric agents. Each complex will have different absorptive characteristics, so the simultaneous determination of individual forms of tocols in a mixture cannot be accomplished by this method.

B. The eight tocols have various reducing potentials and reduce $FeCl_3$ at various rates, requiring the analyst to carefully control the time intervals between mixing and colorimetric determination to prevent inaccurate estimates of the tocols in foods.

C. Compounds other than tocols which have a high reductive potential cause reduction of the $FeCl_3$. This can lead to inaccurate determination of vitamin E.

Chromatography has been used to separate various vitamin E compounds prior to colorimetric analysis. Separation of the eight tocols by 2-dimensional thin layer chromatography has been reported. Kofler et al.

(1972) summarized the adsorbents and eluting solvents commonly used for separation of the tocols. The separated tocols may be visualized by spraying with O-dianisidine or 2,2'-dipyridyl-ferric chloride (Touchstone and Dobbins, 1978). The separated tocols may be quantitated by scraping the spots from the TLC plate, mixing with bipyridyl-ferric chloride reagents, and measuring the absorbance of the colorimetric complex (Waters et al., 1976).

Gas liquid chromatography (GLC) procedures have been developed for quantitation of the tocols commonly found in foods. Wilson et al. (1962) reported separation of eight tocol esters on a 50.4 cm (4 mm i.d.) column packed with 4% SE-30 on silicone-treated celite at 250°C. Rao and Perkins (1972) utilized similar GLC packings to separate the trimethylsilyl ether derivatives of the unsaponifiable fraction of foods. These separated fractions were analyzed by mass spectrometry to confirm the purity of the eluting trimethylsilyl ether derivatives of the eight forms of vitamin E. These GLC procedures required derivatization of the tocols to increase the volatility of the compounds so they can be separated on GLC, extensive sample preparation to remove interfering sterols, triglycerides, and long chain fatty acids, and lengthy analysis time to elute all eight isomers from the GLC column (Wilson et al., 1962 and Abe et al., 1975). The extensive sample preparation may result in the loss of vitamin E during analysis and the long analysis time limits the number of samples which could be analyzed in a single day.

Williams et al. (1972) reported the use of reverse phase high performance liquid chromatography (HPLC) for separation and quantitation of α-tocopherol and α-tocopheryl acetate in a standard mixture of fat soluble vitamins. Von Nieherk (1973) and Abe et al. (1975) determined the

free tocopherols in vegetable oils utilizing HPLC. Both researchers used normal phase adsorption chromatography to separate the four tocopherols in less than 30 minutes per sample. Sample preparation involved dissolving the oil directly into a hexane injection solution, or saponification and injection of the non-saponifiable fraction of oils.

Cavins and Inglett (1974) utilized normal phase adsorption HPLC to separate and quantitate all eight tocols. Separation of the eight tocols using this isocratic method required 105 minutes.

HPLC analysis of d-tocopheryl acetate, the form of vitamin E commonly used to fortify foods, has been reported. Shaikh et al. (1977) and Soderhjelm and Andersson (1978) reported a reverse phase partition chromatography procedure for the analysis of d-tocopheryl acetate in feeds. Shaikh et al. (1977) used a direct methanol extraction method to extract the d-tocopheryl acetate from the feeds, while Soderhjelm and Andersson chromatographed the d-tocopherol in the non-saponified lipid fraction of the feeds.

Widicus and Kirk (1978) reported an HPLC procedure using a normal phase adsorption u-Porasil (Waters Assoc.) column and an 85:15 hexane: chloroform mobile phase for the separation and quantitation of retinyl palmitate and d-tocopheryl acetate directly extracted from ready-to-eat breakfast cereals with chloroform-ethanol-water (20:20:10). Natural occurring d-tocopherol was detectable using this method.

Vitamin E--Biological Activity

Many procedures have been developed to determine the biological activity of vitamin E, as reviewed by Century and Horwitt (1965). The classical method for evaluating vitamin E biological activity is the rat resorption-gestation assay. In this procedure, female rats are placed on

a vitamin E deficient diet, bred, and given the vitamin E test sample shortly before gestation. The female rats are killed just prior to delivery and the litter efficiency is determined.

The dialuric acid hemolysis test assesses the lysis resistance of erythrocytes when exposed to a 0.001M dialuric acid mixture. Erythrocytes from vitamin E deficient animals are more susceptible to degradation by dialuric acid than are erythrocytes from non-deficient animals. Vitamin E deficient rats are fed a test diet and the release of hemoglobin from erythrocytes exposed to dialuric acid may be used to indicate vitamin E activity

Pudelkiewicz and Matterson (1960) reported that the bioavailability of vitamin E may be determined by analyzing the vitamin E content in the livers of chicks fed various sources of vitamin E. This assay requires large concentrations of vitamin E in the diet and lengthy chemical analysis of liver vitamin E.

Kabey and Gilbert (1977) reported a rotifer-based assay for vitamin E activity. Newborn rotifers (Asplanchna sieboldi) are placed onto Petri dishes containing Paramecium aurelia, Penicillin G, and test solutions of vitamin E. The morphology of the rotifers is influenced by the vitamin E activity such that larger doses of vitamin E cause more numerous outgrowths from the rotifer's wall. Statistical analysis of the classification of the rotifer morphology as a function of vitamin E activity allows calculation of vitamin E activity of unknown solutions. This procedure is extremely sensitive (10^{-14}M d-α-tocopherol), consistent, and independent of lipid contamination. The rotifer based assay does not, however, evaluate the digestibility or absorption efficiency which are inherent in animal bioassays.

Machlin et al. (1978) reported a vitamin E rat bioassay that involves measurement of plasma vitamin E levels, pyruvate kinase activity, and aspartate aminotransferase (AspAT) activity. Weanling rats are fed vitamin E deficient diets for approximately 3 weeks. Plasma vitamin E levels are reduced to very low levels and accelerated muscle tissue breakdown causes increased plasma pyruvate kinase and GOT activity. Diets containing test vitamin E are then fed to the depleted rats for 4 days, which causes a dose dependent increase in plasma vitamin E levels and a decrease in plasma pyruvate kinase and AspAT activity.

The determination of the biological activity of α-tocopherol following storage or degradation of α-tocopherol quantitates the actual loss of the vitamin E nutritional quality of the foods. Therefore, bioassays provide an estimate of the vitamin E activity which is more accurate than chemical analysis procedures. Bioassays are generally more expensive, time consuming, difficult, and yield less precise results than chemical analysis.

Degradation Products of α-Tocopherol

The majority of research concerning vitamin E degradation has involved research concerning the reactions of α-tocopherols. This was necessary because of the lack of adequate analytical procedures and the difficulty of determining the mechanism of degradation reactions during degradation of the various foods.

Many oxidation products of α-tocopherol have been isolated and identified. Table 1 summarizes the names, structures, and references which report production of six oxidation products.

Table 1 Names, structures and references reporting preparation of oxidation products of α-tocopherol

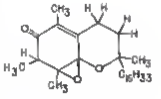
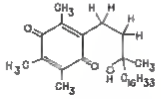
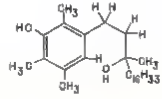
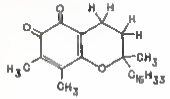
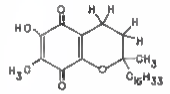
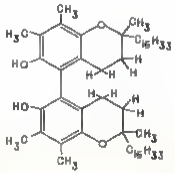
	Name	Reference
	α-tocopheryl oxide	Meyer (1951)
	α-tocopheryl quinone	Karrer and Fritzsche (1938)
	α-tocopheryl hydroquinone	Karrer et al (1938)

Table 1 Continued

	α-tocopheryl red	Smith et al (1939)
	α-tocopheryl purple	Frampton et al (1960)
	α-tocopheryl dimer	Csallany and Draper (1963)

Degradative Reactions of α-Tocopherol

Alpha-tocopherol participates in many reactions which occur during food processing or storage. Fraser et al. (1956) and Moore et al. (1957) reported the destruction of vitamin E by chlorine dioxide, commonly used to bleach wheat flour. Moore et al. reported that untreated flour contained 0.35 mg α-tocopherol/100 g flour while chlorine dioxide-treated flour contained 0.02 mg α-tocopherol/100 g flour. They also reported that untreated flour could supply rats adequate amounts of dietary vitamin E, while chlorine dioxide-treated flours were inadequate sources of vitamin E. The mechanism of α-tocopherol degradation by chlorine dioxide has not been studied extensively.

Oxygen is known to have an effect on α-tocopherol degradation. Fahrenholtz et al. (1974), Foote et al. (1974), and Stevens et al. (1974) reported that α-tocopherol quenched singlet oxygen produced by photooxidation of rubrene or methylene blue. Singlet oxygen is an excited oxygen molecule in which the electrons in the outermost shell are in antiparallel alignment. Fahrenholtz et al. (1974) reported that one mole of α-tocopherol deactivated about 120 moles of singlet oxygen before the α-tocopherol was destroyed, indicating that α-tocopherol may be physically reacting with singlet oxygen.

Metal ions also have been reported to cause oxidation of α-tocopherol. Cort et al. (1978) reported the oxidation of α-tocopherol by ferric and cupric ions. The resulting degradation product was predominately α-tocopheryl quinone. The presence of ferrous or cuprous metal ions were shown not to cause destruction of α-tocopherol. These data suggest α-tocopherol may be oxidized by metal ions only when they are in their higher valence state.

Ormne and Matsue (1976) reported the radical scavenging reaction of α-tocopherol with methyl radicals. In this experiment, methyl radicals were shown to abstract a hydrogen ion from the hydroxyl group of α-tocopherol. Following electron delocalization, the R_1 carbon atom (Figure 1) would become the most reactive and methyl radicals would selectively bond to this site, producing methyl tocopheryl quinone.

Knapp and Teppel (1961) reported the destruction of δ-tocopherol during γ-irradiation. This process was shown to cause a dose dependent destruction of δ-tocopherol. Destruction of δ-tocopherol was more rapid when oxygen was present in solution during irradiation. Although no characteristics of the δ-tocopherol degradation products were reported, one would expect δ-tocopherol to be degraded by a free radical mechanism.

Free radicals, produced during oxidation of unsaturated lipids, react with α-tocopherol and cause oxidation of α-tocopherol. Gruger and Tappel (1970, a and b) examined the degradation of α-tocopherol in ethanol solutions containing α-tocopherol, lipid hydroperoxides, and ferric ions. The following reactions depict the conversion of the lipid hydroperoxides to the active peroxides

$$ROOH + Fe^{+3} \longrightarrow ROO\cdot + H^{+} + Fe^{+2}$$

$$ROOH + Fe^{+2} \longrightarrow RO\cdot + \cdot OH^{-} + Fe^{+3}$$

The free peroxides (ROO· and RO·) reacted with α-tocopherol to form the intermediate, α-tocopheryl oxide, which degraded to α-tocopheryl quinone. It should be noted that α-tocopherol was degraded slowly when only α-tocopherol and ferric ion were combined in solution, but the degradation rate of α-tocopherol was accelerated when lipid hydroperoxides were present. These data indicate two simultaneous reactions may have been occurring.

Degradation of α-Tocopherol During Food Storage

Numerous studies indicate that α-tocopherol may be degraded during the storage of foods through a combination of several reactions occurring simultaneously. It is essential to define the components in foods before generalizing about degradation reactions of α-tocopherol which occur during storage.

Several researchers have reported the loss of α-tocopherol during storage of flours and whole grains. Kuhn et al. (1958) reported that 60% of the tocopherols in whole wheat flour were destroyed during 80 days of storage at 37°C. Kodicek et al. (1959) reported 36% of the α-tocopherol present in whole kernel corn was destroyed after 84 days of storage at room temperature. More recently, Young et al. (1975) reported that the rate of α-tocopherol oxidation was more rapid during storage of high moisture corn than during storage of dry corn. These authors also reported that the peroxide value, an indicator of lipid oxidation, increased with the moisture content of the corn. These data indicate that the moisture content and state of lipid oxidation may affect the rate of α-tocopherol degradation during storage of corn.

Livingston et al. (1968) and Bruhn and Oliver (1978) reported the stability of α-tocopherol during storage of alfalfa and alfalfa meal. Livingston et al. (1968) reported losses of 23, 4, and 28% of the initial α-tocopherol in freeze-dried alfalfa meal at a moisture content of 8.4, 5.4, and 3.3%, respectively, after storage for 84 days at 32°C. Bruhn and Oliver (1978) stored alfalfa in the field and reported that approximately 20% of the α-tocopherol was degraded in 126 days. These authors indicated that these results are of questionable accuracy due to the uncontrolled changes in moisture content, exposure to sunlight, and leaching by rain water.

Jensen (1969) reported that the rate of α-tocopherol degradation in seaweed and seaweed meal was greater at 25% moisture, when compared to samples stored at 10 or 15% moisture. In addition, α-tocopherol in samples stored at 4, 10, 15, or 25°C degraded more rapidly at the higher temperatures. Unfortunately, Jensen did not report the state of lipid oxidation or the metal ion concentration in the seaweed meal.

Kanner et al. (1979) reported the storage stability of α-tocopherol in fresh and dehydrated pepper fruits. Powdered paprika was stored at water activities (a_w) of 0.01 or 0.75 at 37°C. More than 90% of the α-tocopherol present in the paprika was retained during 120 days of storage at 0.75 a_w. Degradation of α-tocopherol was much more rapid when paprika was stored at a a_w of 0.01. The authors attributed the reduction in α-tocopherol degredation at a_w of 0.75 to the solubilization of ascorbic acid, which exhibited an antioxidative effect.

Factors Affecting α-Tocopherol Stability in Foods

From studies concerning the mechanism of α-tocopherol destruction and α-tocopherol loss in foods, data indicate that the stability of α-tocopherol in foods is a function of the type of lipid present, the moisture content, the presence of oxygen, the irradiation dose, the presence of metal ions, and the storage temperature. Because of their primary effect on the stability of α-tocopherol, further discussion of lipid oxidation and moisture content is warranted.

Lipid oxidation. It is known that α-tocopherol is destroyed during autoxidation of unsaturated lipids. Therefore, it would be expected that the storage stability of α-tocopherol is dependent on the rate of lipid oxidation.

A review of the mechanisms of lipid oxidation is presented by Dugan (1976). Lipid oxidation occurs in three stages: (1) the induction phase, (2) the propagation phase, and (3) the termination phase. The induction phase reactions include direct addition of oxygen to a double bond to form a peroxy and a hydrocarbon radical, as well as direct formation of hydroperoxides by reaction with singlet oxygen. The radicals formed during the induction period react with other unsaturated molecules and oxygen to form additional peroxy radicals which causes autoxidation of oxidizable substrates. The termination phase occurs when two radicals react and form non-reactive compounds.

Because of the interaction of α-tocopherol with radicals produced from lipids, the storage stability of α-tocopherol would be expected to be dependent on the rate of lipid oxidation, the rate of peroxide formation, and the mobility of the radicals and α-tocopherol.

Moisture content--water activity. Foods contain various amounts of water. Labuza (1976) provided the following formula for determining the thermodynamic state of water in foods (μ):

$$\mu = \mu_o + RT \ln a_w$$

where μ_o is the standard state chemical potential for water, R is the gas constant, T is the temperature (absolute), and a_w (water activity) is the thermodynamic activity of water, defined as:

$$a_w = \frac{P}{P_o} = \frac{\%ERH}{100}$$

where P is the partial pressure of water in food, Po is the partial pressure of pure water, and %ERH is the equilibrium relative humidity.

A sorption isotherm for a food may be obtained by plotting the a_w of a product as a function of the moisture content at a constant temperature (Figure 2).

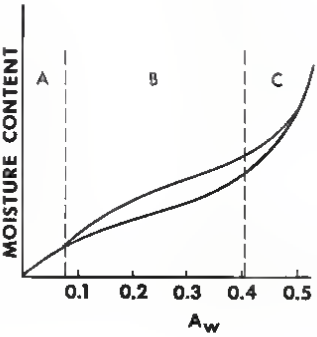


Figure 2. General moisture sorption isotherm for foods exhibiting hysteresis

The moisture content in a food may exhibit different a_ws, depending on whether the sorption isotherm was determined by moisture removal (desorption) or by moisture addition (adsorption). This phenomenon is referred to as hysteresis. Brunauer (1945) identified five types of adsorption isotherms, one of which, the type II, is typical of multilayer formation of water which occurs in dehydrated foods (Labuza, 1968).

Labuza (1968) divided the moisture sorption isotherm into 3 regions (Figure 2). The area described by region A corresponds to the portion of the isotherm below the calculated B.E.T. monomolecular moisture content where the water molecules form a monomolecular layer and are tightly bound and thus not available for reaction. Rockland (1969) reported that the water molecules in this region are bound to hydrophilic sites such as carboxyl and amino groups. Fennema (1976) reported that reactions which are dependent upon water soluble reactions do not occur when the moisture content is in Region A. Region B, the multilayer region, corresponds to the adsorption of water molecules in multilayers. This water is theorized to be loosely bound by hydrogen bonding to hydroxyl and amide groups, and is available for reaction or solubilization of reactants. Region C includes the region where capillary hydration and condensation of water molecules occurs, allowing water to act as a solvent. Physical entrapment and solubility of solutes cause the a_w to approach 1.0 asymptotically.

Labuza (1968) summarized the effects of a_w on degradative reactions occurring in food systems during storage as shown by the stability profile of components in dehydrated foods (Figure 3).

Many theories attempt to relate a_w to the rate of degradation of nutrients during storage. The following discussion will focus primarily on the theories which may be related to α-tocopherol degradation.

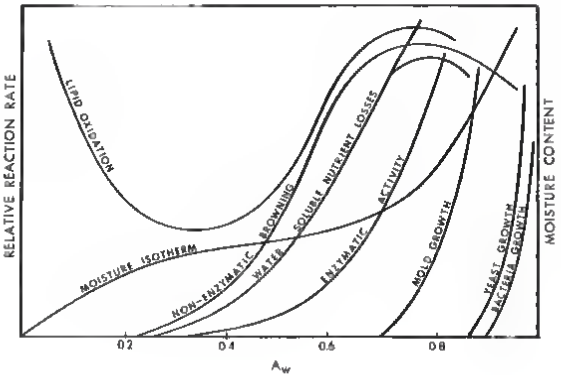


Figure 3 Stability profile of dehydrated foods (from Labuza, 1976).

As discussed previously, the rate of lipid oxidation may be accelerated in the presence of metal ions or oxygen. Lipids are generally more stable towards oxidation at or near the calculated B.E.T. monomolecular moisture content (Selwie, 1959; Maloney et al., 1966; Labuza et al., 1969; and Quast and Karel, 1972). As shown by the lipid oxidation curve in Figure 3, as the a_w is decreased from high values, the rate of lipid oxidation decreases until the a_w approaches or drops below the monolayer value. Decreasing the a_w below the monolayer value causes an increased rate of lipid oxidation. Labuza (1980 a) summarizes the factors affecting lipid oxidation into four theories:

A. As a_w is changed, the hydration of metal catalysts is changed as metal catalysis is effected.

B. The mobility and availability of metal catalysts are altered, which causes changes in the rate of migration of the catalysts to the lipid interface.

C. Peroxide intermediates at the aqueous interface may hydrogen bond to water and not react with oxidizable substrates.

D. The moisture content may change the rate of reaction of free radicals with other species such as proteins.

Salton and Fisher (1957) and Kirk (1976) suggest that the a_w may control the rate of oxygen diffusion through the product and that limited oxygen diffusion may inhibit or slow oxidation reactions.

Kinetic Evaluation of Reactions

Chemical kinetics is the branch of science which studies the rates and patterns of chemical reactions. Laidler (1961) presents an indepth review of the use of chemical kinetics. A brief review of kinetic terminology and interpretation will be presented here.

Four common types of chemical reactions are evaluated by use of either zero, first, pseudo-first, or second order kinetics. The general overall rate equation is:

$$\text{Rate of reaction} = \text{constant} \times [\text{reactant (s)}]^{n}$$

where n is the order of the reaction. Table 2 lists the integrated kinetic equations for simple reactions.

Kinetic data are generated by collecting and analyzing samples for reactant or product concentration after various storage or reaction periods. The data represent the concentration of reactant or product as a function of time. The reaction order may be determined by choosing the kinetic equation (Table 2) which best fits the data, or by use of other graphical or integration methods (Laidler, 1963).

Kinetic evaluation of the storage stability of a nutrient is useful because it provides a model that can be used to describe the storage stability of the nutrient. It also may provide information concerning the reaction mechanism involved in the degradation of the nutrient. However, postulations concerning chemical structure of the reactant species or the transition state cannot be made from simple kinetic evaluation of data.

Thermodynamic Activation Parameters

The Arrhenius equation relates the rate of reaction as a function of temperature according to the following equation:

$$k = A\, e^{Ea/RT}$$

where k is the rate constant, A is an Arrhenius pre-exponential constant, Ea is the activation energy (Kcal mol^{-1}), R is the gas constant (1.987 cal $K^{-1} mol^{-1}$), and T is the temperature (K). Transition state theory

Table 2. Integrated kinetic equations for simple reactions.

Reaction Order	Kinetic Equation
Zero	$C = C_o - kt$
First	$\ln \frac{C}{C_o} = -kt$
Pseudo-first[1]	$\ln \frac{C}{C_o} = -kt$
Second	$\ln \frac{B_o (C)}{C_o (B)} = -k (B_o - C_o) t$

B or C denote concentration of reactant(s) at time t
B_o or C_o denote concentration of reactant(s) at time = 0
t = time of storage
k = rate constant
[1] Pseudo-first order assumes that the reaction is a second order reaction, but the concentration of the second reactant is excessive.

states that Ea is the amount of energy required to form the activated complex from the reactants. If variations in storage parameters do not cause the Ea to change, the energy required to form the activated complex of the reaction is constant. This would suggest that the activated complex is not effected by variations of storage parameters.

Other thermodynamic activation parameters which can be calculated from kinetic data include $\Delta G^{\ddagger}$ (Gibbs free energy of activation), $\Delta H^{\ddagger}$ (enthalpy of activation), and $\Delta S^{\ddagger}$ (entropy of activation). Eyring (1935) reported the absolute reaction rate theory as:

$$k = \frac{k_b T}{h} e^{\Delta S^{\ddagger}} e^{-\Delta H^{\ddagger}/RT}$$

where k is the first order rate constant at temperature T, k_b is the Boltzman constant (1.38×10^{-16} erg K^{-1}), T is the storage temperature (K), h is Plank's constant (6.63×10^{-27} erg sec), R is the gas constant (1.987 cal mol^{-1} K^{-1}), and $\Delta H^{\ddagger} = Ea - RT$. The free energy of activation can be calculated from:

$$\Delta G^{\ddagger} = \Delta H^{\ddagger} - T\Delta S^{\ddagger}$$

If the free energy of activation does not change as a function of the storage parameters, the mechanism of degradation can be assumed not to change.

EXPERIMENTAL PROCEDURES

Composition of the Model Food System

The composition of the model food system used in these studies was similar to that used by Bach (1974) and is reported in Table 3. The non-fat model system contained no added lipids, while the model systems used to determine the effects of saturated or unsaturated fat on the stability of α-tocopherol contained 1% hydrogenated coconut fat or 1% purified methyl linoleate (Privett and Blank, 1962), respectively. The model system was fortified to contain approximately 25 or 50% of the USRDA of α-tocopherol per ounce of dry model system (approximately 125 or 250 μg α-tocopherol per g dry model system).

Preparation of the Model System

The dry components listed in Table 3 were dry blended in a ribbon mixer and stored at 10°C prior to fortification with lipids or α-tocopherol. Portions of the dry blend were slurried into 60°C deionized water to obtain a 40% (w/v) solids slurry. In the non-fat model system, fortification was accomplished by adding the α-tocopherol as an ethanol solution to the model system slurry. In experiments utilizing the model system containing fats, α-tocopherol was mixed with the melted fats (60°C) before addition to the slurry. The model system slurries were homogenized to evenly disperse the lipid soluble components. These homogenized slurries were layered onto stainless steel trays and frozen at -40°C in a freeze dryer for 6-8 hours. The model systems containing no fat or a saturated fat were freeze dried 36 hours at a platen

Table 3. Components of the Model Food System.

Component	% (Dry Wt. Basis)
Powdered Starch[a]	47.7
Corn Syrup Solids[b]	34.0
Soy Protein Isolate[c]	10.2
Sucrose	5.1
Salt (NaCl)	2.0
Fat[d]	1.0

[a] Food Grade, A. E. Staley, Inc.
[b] 15 D.E., Amerimse Maize, Inc.
[c] Promine D, Central Soya C.
[d] Hydrogenated Cocoanut Oil, Durkee Co. or Purified Methyl Linoleate, Sigma Chemical Co.

temperature of 43°C. The model system containing purified methyl linoleate was freeze dried 48 hours at a plateau temperature of 25°C. The freeze dried samples were removed from the freeze dryer under a nitrogen atmosphere, ground to a fine powder in a Waring blender in an air atmosphere, and equilibrated to various water activities (a_w).

A_w Equilibration

All experiments in this study were performed on the adsorption leg of the sorption hysteresis loop. The dry model food system containing no fat was equilibrated to water activities of 0.10, 0.24, 0.40 or 0.65 at 20, 30 and 37°C by forcing conditioned air provided by an Aminco-Aire unit through an equilibration chamber containing the dry model system, as previously reported by Rach (1974). Moisture equilibration in the model system was achieved in 24 hours. The model systems containing lipids were equilibrated to water activities of 0.11, 0.23, 0.42 and 0.67 at 20, 30 and 37°C by placing the powdered model system into desiccators containing various saturated salt solutions to provide the desired relative humidity (Rockland, 1960). The desiccators were evacuated (685 mm Hg vacuum) and stored at the appropriate temperature for 24 hours, prior to releasing the vacuum by drawing air through an appropriate saturated salt solution. The a_w of the equilibrated model systems was confirmed by vapor pressure manometry, as described by Lewicki et al. (1978).

Packaging and Storage of Equilibrated Model Systems

To prevent shifts in the moisture content, the equilibrated model food systems were packaged in cans lined with c-enamel, an oleoresinous coating. Portions of the model food system weighing approximately 15 g were sealed into either 303 x 406 cans (303 cans) or 208 x 006 cans (TDT

cans). Theoretical calculations show that the 303 cans contained approximately 4.8 mmol of O_2 and as much are referred to as the excess headspace containers. The TDT cans were packed tightly with product allowing no free headspace. The oxygen content in the TDT cans was estimated to be 0.05 mmol of oxygen. The equilibrated samples were stored isothermally at 20, 30 and 37°C.

Iron and Copper Determination

Total iron and copper content of the model system was determined utilizing atomic absorption spectroscopy. Approximately 5.00 g of model system was refluxed with 20 ml 50% (v/v) HCl for 5 min. After dilution with 50% (v/v) HCl, the samples were filtered through Whatman #1 filter paper. Sample filtrates and appropriate standards were assayed for iron and copper using a Model 460 Perkin Elmer Atomic Absorbance spectrophotometer.

α-Tocopherol Determination

Alpha-tocopherol was quantitatively determined by a high performance liquid chromatographic (HPLC) procedure reported by Widicus and Kirk (1979). Approximately 4 g of model system, accurately weighed to the nearest 0.1 milligram, was extracted with a 4:2:1 chloroform-ethanol-water mixture. In the experiments using a model system containing unsaturated fats, 0.002 g BHT was added to inhibit oxidation during the extraction procedure. After filtering, the solvent was evaporated to dryness, and the lipid extract was dissolved in an aliquot of the HPLC mobile phase (85 + 15 hexane-chloroform). An aliquot of the lipid extract was injected onto a HPLC μ-Porasil (Waters Assoc.) column which was isocratically eluted at 1.5 ml/min. Absorbance at 280 nm was monitored, and quantitation of the eluting peak was accomplished by peak

height measurement. Extraction efficiency was continually monitored by adding known amounts of α-tocopherol to the model system during extraction and calculating the recovery of the added α-tocopherol.

Methyl Linoleate Determination

Methyl linoleate was determined by a gas-liquid chromatography (GLC) procedure. Approximately 5 g of model system, accurately weighed to the nearest 0.01 g, was placed into a 30 ml screw cap test tube. Twenty-five milliliters of a 2:1 chloroform-methanol solution containing 0.002 g BHT was added to the tube before sealing. The tubes were placed horizontally in a shaking bath oscillating at 120 strokes per minute at room temperature. After 30 minutes of extraction, the extract was filtered through Na_2SO_4 on Whatman #2 filter paper. Aliquots of the filtered extract were analyzed by gas chromatography. The following conditions were established for the GLC analysis of the lipid extract:

Gas chromatograph ——— Tracor 550

Temperature ——— 175°C

Detector ——— Flame Ionization

Column data

Flow rates.

carrier gas (nitrogen) ——— 40 ml/min.

hydrogen ——— 40 ml/min.

air ——— 460 ml/min.

Packing ——— 10% EGSSX on Gas Chrom Q

Length ——— 144 cm

Internal diameter ——— 4 mm

Oxygen Uptake Measurements

Approximately 2 g of the model systems, accurately weighed to the nearest 0.1 mg, containing unsaturated fat were weighed into 18 ml respirometer flasks. Following moisture equilibration in vacuum desiccators, the respirometer flasks were connected to a pre-conditioned constant pressure respirometer (Gilson Medical Instruments, Model IGP14). The 150 ml reference flask headspace volume was adjusted by addition of the saturated salt solution used to equilibrate the a_w of the samples. Oxygen uptake was monitored until no further oxygen consumption was detected. Blank samples (equilibrated model systems containing no fat or α-tocopherol) were prepared and monitored with the test samples. The amount of oxygen consumed or expired by the blank samples was subtracted or added to the test samples to adjust for small changes in the storage temperature. The oxygen uptake by the model systems containing methyl linolenate was calculated to obtain the rate of O_2 taken up per mole of methyl linolenate present prior to storage.

Biological Activity Determination

The biological activity of α-tocopherol following storage was evaluated by a rat bioassay similar to that reported by Machlin et al. (1978). Model systems containing no fat, hydrogenated coconut fat or methyl linolenate were stored until approximately 75% of the initial α-tocopherol had degraded. All samples were stored at -30°C until needed for the rat bioassay.

Male weanling Sprague-Dawley rats were caged individually in stainless steel cages at 24°C and fed a vitamin E depletion diet (Table 4) for 48 days. Each rat was given food only during the 12 hour dark period of each day. Following the 48 day depletion periods, the rats were randomly

Table 4. Components of the vitamin E depletion diet fed to weanling rats for 48 days prior to the α-tocopherol bioassay.

Ingredients	%
Glucose Monohydrate[a]	62.8
Casein[b]	20.0
Ethyl Linoleate[c]	6.0
Salts[d]	4.0
Alphacel[e]	3.0
Vitamin Mix[f]	2.2
Corn Oil[g]	2.0

(a) Teklad Test Diets, Inc., (b) vitamin free - ICN Nutritional Biochemicals, (c) 75%, with 0.02% BHA, United States Biochemical Corp., (d) salt mixture Draper 4164, United States Biochemical Corp., (e) ICN Nutritional Biochemicals, (f) void of α-tocopherol, ICN Nutritional Biochemicals, (g) tocopherol stripped, United States Biochemical Corp.

assigned to groups of 8 or 9 rats per group. Model feed systems containing no fat and containing various levels of α-tocopherol were prepared immediately prior to the end of the rat depletion period. The fresh model systems and the equilibrated stored model systems were blended with the depletion diet to obtain the standard and test diets which were fed to the rats for four days.

Blood was collected into a heparinized tube directly from the cervical vessels following decapitation of the rat. The blood was centrifuged and the plasma separated from the red blood cells. Plasma samples were stored at −10°C in sealed glass test tubes until assayed for plasma α-tocopherol, plasma aspartate aminotransferase (AspAT) (E.C. 2.6.1.1), or pyruvate kinase (PK) (E.C. 2.7.1.40) activity.

Plasma α-tocopherol was determined by extracting a 1:1 mixture of plasma and ethanol with 1 part hexane. Following centrifugation, an aliquot of the hexane layer was injected onto a μ-Porasil HPLC column using the same procedure as described previously.

Plasma AspAT activity was determined by use of a premixed test kit (Sigma Chemical Co.) which measured the rate of loss of NADH in the coupled reactions

$$\text{Aspartic Acid} + \alpha\text{-Ketoglutaric Acid} \xrightarrow{\text{AspAT}} \text{Oxalacetic Acid} + \text{Glutamic Acid}$$

$$\text{Oxalacetic Acid} + \text{NADH} \xrightarrow{\text{MDH}} \text{Malic Acid} + \text{NAD}$$

where MDH denotes malate dehydrogenase, NADH denotes reduced nicotinamide adenine dinucleotide, and NAD denotes oxidized nicotinamide adenine dinucleotide.

The rate of NADH loss at 25°C was monitored spectrophotometrically at 340 nm utilizing a Gilford spectrophotometer (Gilford Instrument Co.,

lac., Model 250). Activity was calculated as International Units/liter (μmol aspartic acid converted to oxaloacetic acid per minute per liter).

Plasma PK activity was determined by the analysis reported by Boehringer Mannheim Co. (1973). The rate of NADH oxidation was monitored in the coupled reactions:

$$\text{Phosphoenolpyruvate} + \text{ADP} \xrightarrow[Mg^{2+},\ K^{+}]{PK} \text{Pyruvate} + \text{ATP}$$

$$\text{Pyruvate} + \text{NADH} + H^{+} \xrightarrow{LDH} \text{L-Lactate} + \text{NAD}^{+}$$

where LDH denotes lactate dehydrogenase.

The loss of NADH at 25°C was followed spectrophotometrically (340 nm) in a Gilford spectrophotometer (Gilford Instrument Co., Inc., Model 250). Activity was reported as units/ml plasma (μmol phosphoenol pyruvate converted to pyruvate per minute per ml plasma).

Synthesis of α-Tocopherol Degradation Products

Several oxidation products of α-tocopherol were prepared following previously published procedures. α-Tocopheryl oxide was synthesized by reacting α-tocopherol with $FeCl_3$ in the presence of 2,2'-bipyridine as described by Boyer (1951). α-Tocopheryl quinone was prepared by acidifying α-tocopherol oxide with HCl (Boyer, 1951). α-Tocopherol dimer and trimer were synthesized by reacting α-tocopherol with $K_3Fe(CN)_6$ in NaOH (Csallany and Draper, 1962). Ultraviolet absorption scans of α-tocopherol oxidation products were run and compared to published data to ensure purity of these compounds. α-Tocopherol dimer was purified by collecting only the yellow dimer peak eluting from a preparative normal phase HPLC column. The purified α-tocopheryl oxide, α-tocopheryl quinone, and α-tocopheryl dimer were injected onto the analytical μ-Porasil HPCL column used for determination of α-tocopherol and the HPLC characteristics were determined.

Data Analysis

The degradation rates of α-tocopherol and methyl linolenate were calculated by fitting the data to the kinetic equations in Table 2. SAS computer programs (Barr et al., 1979) utilizing least squares linear regression techniques were used to calculate the slope, the correlation coefficients, and the standard deviations of the kinetic equations. The order of the reaction was determined by choosing the equation which resulted in the highest correlation coefficient and best graphical model of the data.

RESULTS

The storage stability of α-tocopherol in a dehydrated model food system was evaluated. The model food system was formulated with either no fat, saturated fat, or unsaturated lipids. These model systems were equilibrated to water activities (a_w) of approximately 0.10, 0.24, 0.40 or 0.65 at 20, 30 and 37°C. The volume of headspace air in the storage containers was varied by use of two different sized enameled metal cans. The stability of α-tocopherol was evaluated by measuring the loss of α-tocopherol as a function of time. The biological activity of α-tocopherol after storage in the model system was evaluated by a rat bioassay.

α-Tocopherol Stability: No Fat Model System

The degradation of α-tocopherol in a model food system containing no fat stored at various a_ws, temperatures, and oxygen to α-tocopherol molar ratios was studied. The storage parameters included: water activities of 0.10, 0.24, 0.40, or 0.65; storage temperatures of 20, 30, or 37°C; and 1450 moles O_2 per mole α-tocopherol (303 can) or 15 moles O_2 per mole α-tocopherol(TDT can). A nonfat model food system was chosen in order to characterize the nonlipid mediated degradation of α-tocopherol in a simulated food system.

All of the model food systems in this study were stored until a minimum of 87.5% of the initial α-tocopherol content had degraded, except for the samples where the half-life of α-tocopherol was greater than 150 days. In these cases, model systems were stored until 75% of the initial α-tocopherol content had degraded. In most cases, this was

equivalent to the loss of α-tocopherol through three half-lives which permitted valid kinetic modeling of the loss of α-tocopherol.

The loss of α-tocopherol could be satisfactorily described by the integrated first order kinetic model (Table 2). First order degradation plots describing α-tocopherol loss during storage of the model system at 37°C in 303 cans at various a_w are shown in Figure 4. Alpha-tocopherol losses in the non-fat model system were described by first order kinetic equation using linear regression analysis. The slopes of the regression equations (the first order rate constants) are shown in Table 5. All of the calculated rate constants were significantly greater than 0 ($p \leq 0.0014$), had correlation coefficients ($|R|$) ≥ 0.90, and had standard deviations less than 10% of the reported rate constants. Half-lives of α-tocopherol loss were calculated from:

$$t_{\frac{1}{2}} = \frac{0.693}{k}$$

where $t_{\frac{1}{2}}$ denotes the half-life (days) and k denotes the first order rate constant (day^{-1}).

The first order rate constants and half-lives for α-tocopherol degradation were affected by the a_w, the storage temperature, and the oxygen content in the containers. Quadratic prediction equations for the first order rate constants and half-lives of α-tocopherol loss as a function of a_w and temperature were calculated and are listed in Table 5. The prediction equations indicate that the rate of α-tocopherol loss was lowest at low a_w and increased as the a_w was increased. The rate of α-tocopherol loss increased with increasing storage temperature. According to the prediction equations of the first order rate constants, an interaction between the a_w and storage temperatures was present, indicating the magnitude of the effect of increasing the a_w was not the same at each storage temperature.

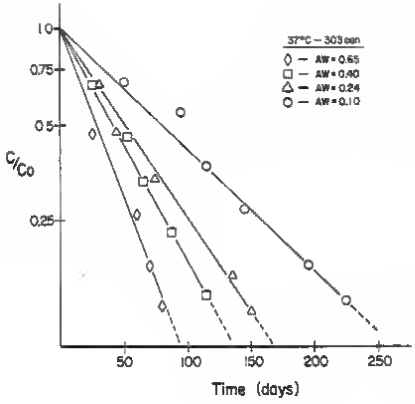


Figure 4. First order plot of α-tocopherol degradation in a dehydrated model food system stored at 37°C in a 303 can at various water activities

Table 5. First order rate constants, half-lives, and prediction equations for α-tocopherol degradation in a dehydrated model food system containing no fat at various water activities, storage temperatures, and storage container oxygen contents.

Temp. (°C)	a_w	303 Can[a] k^c	303 Can[a] $t_{1/4}{}^d$	TDT Can[b] k^c	TDT Can[b] $t_{1/4}{}^d$
37	0.65	15.94	43.5	13.40	51.7
	0.40	14.85	46.7	13.15	52.7
	0.24	12.84	53.9	11.38	60.9
	0.10	8.02	86.4	8.63	80.3
30	0.65	7.11	97.5	6.49	106.8
	0.40	6.28	110.4	6.03	114.9
	0.24	6.22	111.4	5.92	117.3
	0.10	4.97	139.4	4.70	147.5
20	0.65	5.18	133.8	5.54	125.1
	0.40	5.23	132.5	4.53	152.9
	0.24	4.47	155.0	3.26	212.6
	0.10	3.24	213.9	3.23	214.6

Prediction equations (and standard error of coefficient estimates):

$$k^c_{303} = \underset{(7.65)}{31.04} + \underset{(9.64)^y}{4.86\, a_w} - \underset{(0.54)}{2.40T} + \underset{(0.22)^y}{0.55\, a_w T} - \underset{(9.29)^y}{18.47\, a_w^2} + \underset{(0.01)}{0.05\, T^2} \quad R^2=0.965$$

$$t^d_{\frac{1}{4},303} = \underset{(74.91)}{218.8} - \underset{(94.36)^y}{448.2\, a_w} + \underset{(5.33)}{5.22T} - \underset{(2.14)^y}{3.93\, a_w T} + \underset{(90.97)^y}{320.4\, a_w^2} - \underset{(0.09)}{0.22\, T^2} \quad R^2=0.975$$

$$k^c_{TDT} = \underset{(5.69)}{23.63} + \underset{(7.17)^y}{7.49\, a_w} - \underset{(0.40)}{1.88\, T} + \underset{(0.16)^y}{0.19\, a_w T} - \underset{(6.91)^y}{10.23\, a_w^2} + \underset{(0.01)}{0.04\, T^2} \quad R^2=0.973$$

$$t^d_{\frac{1}{4},TDT} = \underset{(84.87)}{303.2} - \underset{(106.9)^y}{429.8\, a_w} + \underset{(6.04)}{0.96\, T} + \underset{(2.43)^y}{8.03\, a_w T} + \underset{(103.1)^y}{130.1\, a_w^2} - \underset{(0.11)}{0.18\, T^2} \quad R^2=0.975$$

[a] 1450:1 Calculated molar ratio of oxygen:α-tocopherol

[b] 15:1 Calculated molar ratio of oxygen:α-tocopherol

[c] First order rate constant, $x10^{-3}$ day^{-1}

[d] Half-life, day

The effect of storage temperature on the degradation rate of α-tocopherol in a model system containing no fat could be described by the Arrhenius equation (Table 6). The apparent activation energies (Ea) for α-tocopherol degradation in a model food system stored at various a_ws or storage container oxygen contents ranged from 8.9 to 13.1 kcal mol^{-1} (Table 6). No significant differences($p \leq 0.01$) between any of the experimental activation energies were found.

The thermodynamic activation parameters $\Delta G^{\ddagger}$, $\Delta S^{\ddagger}$, and $\Delta S^{\ddagger}$ were calculated from the first order rate constants and the apparent activation energies and are reported in Table 7. Neither the enthalpy ($\Delta H^{\ddagger}$) nor entropy ($\Delta S^{\ddagger}$) of activation were linearly dependent on the a_w of the sample or the oxygen content in the storage container. Gibbs free energy of activation ($\Delta G^{\ddagger}$) ranged from 27.1 to 27.4 through the a_w range of 0.10 to 0.65. Linear and quadratic equations for $\Delta G^{\ddagger}$ as a function of a_w (Table 7) indicate that $\Delta G^{\ddagger}$ is affected by a_w.

The volume of headspace present in the storage containers affected the stability of α-tocopherol in the model food system. The observed rate constants of α-tocopherol degradation were generally greater when the molar ratio of oxygen to α-tocopherol in the container was 1450:1 (303 cans) rather than 15:1 (TDT cans). The rate constants of α-tocopherol degradation in the model food system in 303 cans were significantly greater than those from storage in TDT cans at 37°C and a_ws of 0.65 ($p \leq 0.01$), 0.42 ($p \leq 0.05$), or 0.24 ($p \leq 0.05$) (Figure 5). Differences between the rate constants of α-tocopherol degradation in 303 cans or TDT cans were not as pronounced at 30 or 20°C, indicating a temperature-a_w-storage container interaction. A paired comparison t-test showed that the rate constants, independent of a_w or temperature, were

Table 6. Apparent activation energies for α-tocopherol destruction in a dehydrated model food system containing no fat stored at various water activities and storage container oxygen contents.

Water Activity	Apparent Activation Energy, Kcal mol^{-1}	
	303 Cm[a]	TDT Cm[b]
0.65	11.4	8.9
0.40	10.5	10.8
0.24	10.8	13.1
0.10	9.5	10.1

[a] 1450:1 Calculated molar ratio of oxygen:α-tocopherol
[b] 15:1 Calculated molar ratio of oxygen:α-tocopherol

Table 7. Thermodynamic activation parameters and prediction equations for α-tocopherol degradation in a dehydrated model food system containing no fat stored at various water activities and storage container oxygen contents.

a_w	$\Delta H^{\ddagger}$ (kcal mol^{-1})	$\Delta S^{\ddagger}$ (kcal mol^{-1} K^{-1})	$\Delta G^{\ddagger}$ (kcal mol^{-1})
		303 Can[a]	
0.65	10.8	-54.6	27.1
0.40	9.9	-57.8	27.1
0.24	10.2	-57.1	27.2
0.10	8.9	-62.3	27.4
		TDT Can[b]	
0.65	8.3	-63.2	27.1
0.40	10.2	-56.9	27.2
0.24	12.5	-50.0	27.4
0.10	9.5	-60.0	27.4

Prediction equations (and standard error of coefficient estimates):

$$\Delta G^{\ddagger}_{303} = 27.38 - 0.509\, a_w \qquad R^2 = 0.85$$

$$= \underset{(0.026)}{27.57} - \underset{(0.168)}{1.97}\, a_w + \underset{(0.216)}{1.92}\, a_w^2 \qquad R^2 = 0.99$$

$$\Delta G^{\ddagger}_{TDT} = 27.48 - 0.606\, a_w \qquad R^2 = 0.85$$

$$= \underset{(0.143)}{27.48} - \underset{(0.917)}{0.57}\, a_w - \underset{(1.16)}{0.044}\, a_w^2 \qquad R^2 = 0.91$$

[a] 1450:1 Calculated molar ratio of oxygen:α-tocopherol
[b] 15:1 Calculated molar ratio of oxygen:α-tocopherol

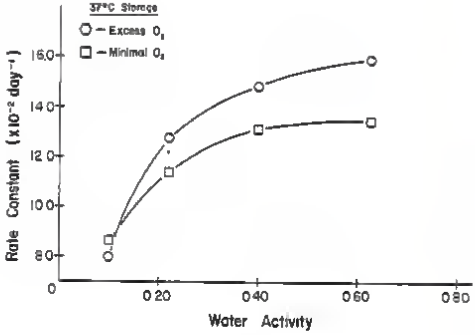


Figure 5. First order rate constants for degradation of α-tocopherol in a model food system containing no fat stored at 37°C as a function of water activity at oxygen to α-tocopherol molar ratios of 1450:1 (Excess O_2) or 15:1 (Minimal O_2).

significantly greater ($p<0.05$) when the model system was stored in 303 cans as compared to the TPT cans.

High performance liquid chromatograms of the lipids extracted from the model system before and after storage are shown in Figures 6 and 7. Recovery studies, which involved quantitation of α-tocopherol added during the extraction, indicated that the extraction of α-tocopherol was consistently >90% throughout the storage study. Several additional peaks were detected following storage of the α-tocopherol fortified model system which contained no fat. The peak labelled TO had a retention time characteristic of α-tocopherol oxide, while the peak labelled TQ had a retention time characteristic of α-tocopheryl quinone. The absorbance ratios of the peaks believed to be α-tocopheryl oxide and α-tocopheryl quinone relative to the α-tocopherol peak increased when monitored at 254 nm rather than at 280 nm. Alpha-tocopheryl oxide and α-tocopheryl quinone were determined to have absorbance maximums at 250 and 264 nm, respectively. The relative increased absorbance of the peaks labelled TO and TQ at 254 nm support the tentative identification of these compounds as α-tocopheryl oxide and α-tocopheryl quinone. Further analysis of these compounds was not done.

α-Tocopherol Stability: Saturated Fat Model System

The loss of α-tocopherol in a dehydrated model food system containing saturated lipids was studied. Model system containing 1% hydrogenated coconut oil was equilibrated at 37°C to a_w of 0.11, 0.23, 0.43, and 0.67 and stored in 303 cans which contained 4.8 cmol oxygen. The concentration of α-tocopherol in the model systems was monitored until less than 25% of the original α-tocopherol remained (Figure 8).

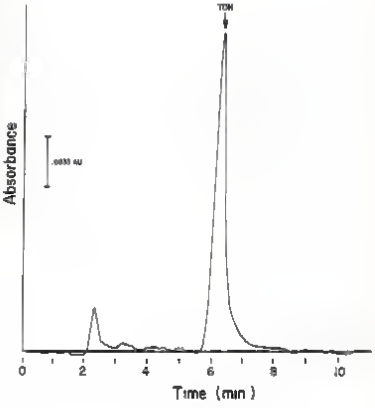


Figure 6. Typical HPLC chromatogram of the extract from a model food system containing no fat obtained at the initiation of a storage period. TOH denotes the peak characteristic of α-tocopherol. Absorbance was monitored at 280 nm

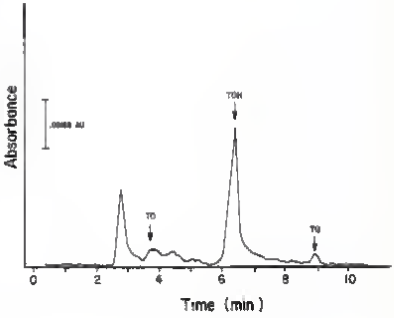


Figure 7 Typical HPLC chromatogram of the extract from a model food system containing no fat obtained after degradation of approximately one-half of the initial α-tocopherol content. TO denotes α-tocopheryl oxide, TOH denotes α-tocopherol, and TQ denotes α-tocopheryl quinone

Figure 8. First order plot of α-tocopherol degradation in a dehydrated model food system containing hydrogenated coconut fat stored at 37°C in 303 cans at various water activities.

The loss of α-tocopherol was described by first order kinetics. All of the slopes were >0 ($p \leq 0.0007$) with correlation coefficients ($|R|$) ≥ 0.93. The first order rate constants and half-lives describing α-tocopherol loss during storage in a model food system containing a saturated fat are reported in Table 8. The rate of α-tocopherol loss was a quadratic function of the a_w, as shown by the prediction equation in Table 8. The rate of α-tocopherol degradation was not significantly different ($p \leq 0.01$) when the a_w was 0.67 or 0.42. However, these degradation rates were significantly lower ($p \leq 0.05$) when the a_w was decreased to 0.23 or 0.11.

α-Tocopherol Stability Unsaturated Fat Model System

Alpha-Tocopherol degradation was monitored during storage of a model food system containing an unsaturated lipid, methyl linoleate. The model food system was stored isothermally at 20, 30 or 37°C at a_ws of 0.11, 0.23, 0.43, or 0.67 in containers which contained 4.8 or 0.05 mmol oxygen.

HPLC chromatograms of the lipid extract from the model system containing unsaturated fat, sampled prior to and during storage, are shown in Figures 9 and 10. Data in these figures show that during storage, the concentration of methyl linoleate (ML) and α-tocopherol (TOH) decreased. Initially, α-tocopherol was extracted from the model system with $CHCl_3$, ethanol, and water. The percent recovery of α-tocopherol added during the extraction procedure decreased from 95% before storage of the model system to 45% after storage of the model system for several weeks. BHT (0.002 g) was added to each flask to prevent oxidation of α-tocopherol during extraction. As a result of this modification of the extraction procedure, the recoveries of α-tocopherol added during the extraction procedure remained ≤90% throughout all storage studies.

Table 8. First order rate constants, half-lives, and prediction equations for the degradation of α-tocopherol in a dehydrated model food system containing saturated lipids stored at 37°C in 303 cans at various water activities.

a_w	k^a	$t_{\frac{1}{2}}^b$
0.67	15.2	42.8
0.43	14.8	46.8
0.23	13.1	52.9
0.11	9.6	72.2

Prediction equation (and standard error of coefficient estimates):

$$\hat{k}^a = \underset{(1.33)}{6.62} + \underset{(8.41)}{32.8}\, a_w - \underset{(10.5)}{30.0}\, a_w^2 \qquad R^2 = 0.97$$

[a] First order rate constant ($\times 10^{-3}$ day^{-1})

[b] Half-life, days

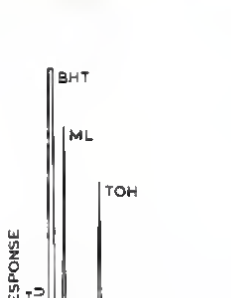


Figure 5 Typical HPLC chromatogram of the extract from a model system containing methyl linoleate and α-tocopherol prior to storage. BHT denotes butylated hydroxytoulene added during extraction procedure, ML denotes methyl linoleate, and TOH denotes α-tocopherol.

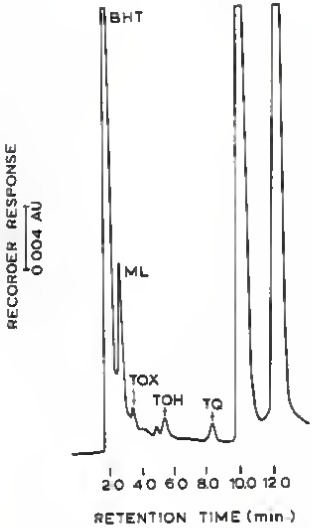


Figure 10. Typical HPLC chromatogram of the extract from a model food system containing α-tocopherol and methyl linoleate obtained after storage until approximately 87.5% of the initial α-tocopherol had degraded. BHT denotes butylated hydroxy-toulene added during extraction, ML denotes methyl linoleate, TOX denotes α-tocopheryl oxide, TOH denotes α-tocopherol, and TQ denotes α-tocopheryl quinone.

Peaks with retention times characteristic of α-tocopheryl oxide (TOX) and α-tocopheryl quinone (TQ) appeared during storage. The two large peaks eluting at 10.5 and 12 minutes were not identified.

Alpha-tocopherol loss in the model system containing methyl linoleate fit zero order reaction kinetics (Figure 11) with rates significantly greater than 0 ($p \leq 0.002$), correlation coefficients ($|R|$) ≥ 0.88, and standard deviations from 4 to 13% of the reported rate constants. The zero order rate constants for α-tocopherol degradation at various temperatures, a_ws, and storage container oxygen contents are shown in Tables 9 and 10.

As shown by the plot of data in Figure 11, the a_w of the model food system appeared to affect the rate of α-tocopherol degradation. The degradation rate of α-tocopherol was the slowest at an a_w of 0.23. As the a_w was increased or decreased from 0.23, the rate of α-tocopherol degradation increased. The rate of α-tocopherol degradation was greater at a_w of 0.43 than an a_w of 0.67. Linear prediction equations for the α-tocopherol zero order rate constants are reported in Tables 9 and 10. These equations indicate that the zero order rate constants were not significantly affected ($p \leq 0.05$) by a_w. Differences between the rate constants at a_w of 0.42 and at each of the other a_ws over both container types and storage temperatures were on the average significantly greater than zero ($p \leq 0.01$) implying the rate loss was greatest at a_w of 0.42. The average difference in the rate constants for the a_w levels of 0.67 and 0.11 was not different from zero. The rate constants at a_w of 0.67 and 0.11 were significantly greater, on the average, than the rate constants at a_w of 0.23. Cubic prediction equations for the zero order rate constants as a function of a_w and temperature were calculated and

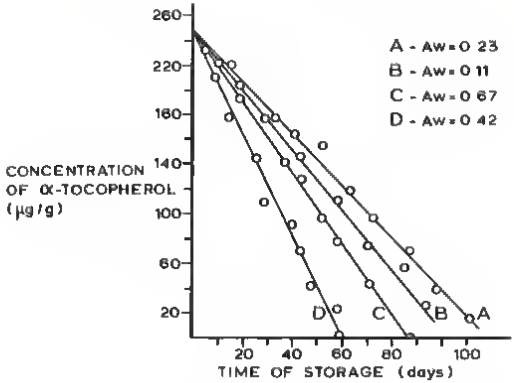


Figura 11. Zero order plot of α-tocopherol degradation during storage in a model food system containing methyl linoleate stored in a 303 cm at 30°C at various water activities.

Table 9. Zero order rate constants[a] and prediction equations for α-tocopheral degradation in a model food system containing 1% methyl linoleate and 125 μg α-tocopherol per g model system, stored at various water activities, storage temperatures, and storage container oxygen contents.

	Storage Temperature (°C)					
	37		30		20	
	TDT[b]	303[c]	TDT[b]	303[c]	TDT[b]	303[c]
Water Activity	Can	Can	Can	Can	Can	Can
0.67	2.88	2.78	2.09	1.74	0.58	0.54
0.42	2.43	3.46	2.82	1.83	1.39	1.28
0.23	1.90	1.70	1.86	1.50	0.52	0.52
0.11	2.30	2.22	2.33	1.42	0.77	0.55

Prediction equations (and standard error of coefficient estimates)

$\hat{k}^a_{TDT}$ = −1.13 + 0.497 a_w + 0.096 T $\quad R^2 = 0.72$
(0.643) (0.661)[w] (0.020)

= 0.646 − 21.9 a_w + 0.097 T + 72.4 a_w^2 + 64.5 a_w^3 $\quad R^2 = 0.83$
(1.25) (12.6)[w] (0.0179) (38.0)[w] (32.7)[w]

$\hat{k}^a_{303}$ = −1.79 + 0.895 a_w + 0.106 T $\quad R^2 = 0.79$
(0.607) (0.624)[w] (0.019)

= 0.555 − 21.3 a_w + 0.097 T + 69.8 a_w^2 − 62.0 a_w^3 $\quad R^2 = 0.83$
(1.24) (12.6)[w] (0.0179) (38.0)[w] (32.7)[w]

[a]Zero order rate constants, μg α-tocopherol day^{-1}
[b]Contained 0.05 mmol gaseous O_2
[c]Contained 4.8 mmol gaseous O_2

Table 10. Zero order rate constants[a] and prediction equations for α-tocopherol degradation in a model food system containing 1% methyl linoleate and 250 μg α-tocopherol per g model system stored at various water activities, storage temperatures, and storage container oxygen contents.

	Storage Temperature (°C)					
	37		30		20	
Water Activity	TDT[b]	303[c]	TDT[b]	303[c]	TDT[b]	303[c]
0.67	6.04	4.95	3.31	3.05	0.98	0.97
0.42	6.11	4.38	4.22	3.62	2.38	2.00
0.23	4.24	3.58	3.53	2.12	1.46	0.96
0.11	5.34	3.69	3.71	2.75	1.37	0.68

Prediction equations (and standard error of coefficient estimates):

$\hat{k}_{TDT}$ = −3.22 + 0.496 a_w + 0.228 T R^2 = 0.89
(0.849) (0.723)[W] (0.0265)

= 0.646 − 21.9 a_w + 0.0956 T + 72.4 a_w^2 − 64.5 a_w^3 R^2 = 0.93
(1.25) (12.6)[W] (0.0179) (38.0)[W] (32.7)[W]

$\hat{k}_{303}$ = −2.92 + 1.52 a_w + 0.176 T R^2 = 0.90
(0.652) (0.670)[W] (0.0203)

= −1.26 − 20.9 a_w + 0.176 T + 76.4 a_w^2 − 70.3 a_w^3 R^2 = 0.96
(1.06) (10.8)[W] (0.0153) (32.5)[W] (27.9)[W]

[a] Zero order rate constants, μg α-tocopherol day^{-1}
[b] Contained 0.05 mmol gaseous O_2
[c] Contained 4.8 mmol gaseous O_2

are shown in Table 9. Since these models are cubic and are fitted to only four levels of a_w, the equation goes nearly through the average response at the four points. The goodness of fit of the equation is determined by noticing the increased value of R^2 with the cubic equation relative to fitting only the linear first-degree equation.

The amount of headspace present in the storage container did affect the rate of α-tocopherol degradation. The rate of α-tocopherol loss in model systems stored in the TDT cans (contained 0.05 mmol O_2) was significantly greater ($p<0.01$) than the rate of α-tocopherol loss in the model systems stored in the 303 cans (contained 4.8 mmol O_2) when tested with a paired comparison t-test. Data in Figure 12 shows that the α-tocopherol concentration decreased to approximately 0 μg/g in the model system stored with 4.8 m mol O_2. In contrast, the α-tocopherol concentration in the model system stored with 0.05 mmol O_2 decreased to about 20 μg/g and remained constant.

The rate of α-tocopherol degradation was dependent on the initial concentration of α-tocopherol (Tables 9 and 10). The zero order rate constants were significantly larger ($p<0.01$) when the initial concentration of α-tocopherol was increased from 125 to 250 μg α-tocopherol per g model system.

A significant ($p<0.05$) temperature dependence of α-tocopherol degradation in a model system containing methyl linolenate was shown by the prediction equations (Tables 9 and 10). The temperature dependance was described by the Arrhenius equation. Apparent Ea's for α-tocopherol loss are shown in Table 11. Water activity and Ea were not linearly related and no Ea could be shown to be significantly different from the others at $p<0.05$.

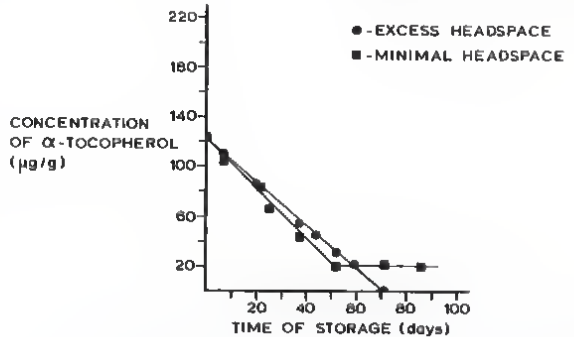


Figure 12. Zero order plot of α-tocopherol degradation during storage in a model food system containing methyl linoleate stored at 30°C at a a_w of 0.67 with an excess headspace (303 can containing 4.5 mmol gaseous O_2) or minimal headspace (TDT can containing 0.05 mmol gaseous O_2).

Table 11. Apparent activation energies[a] for degradation of α-tocopherol in a model food system containing 1% methyl linoleate stored at various initial α-tocopherol concentrations, water activities, and storage container oxygen contents.

	α-Tocopherol Concentration			
	125 μg α-tocopherol per g		250 μg α-tocopherol per g	
Water Activity	TDT[b] Cae	303[c] Can	TDT[b] Can	303[c] Can
0.67	15.6	18.7	19.4	17.5
0.42	6.0	10.2	10.1	8.5
0.23	14.4	13.1	11.7	14.3
0.11	11.0	15.0	14.7	18.2

[a]Apparent activation energies, kcal mol^{-1}
[b]Contained 0.05 mmol gaseous O_2
[c]Contained 4.8 mmol gaseous O_2

Methyl Linoleate Stability - Unsaturated Fat Model System

The stability of methyl linoleate in a dehydrated model food system containing 125 or 250 µg α-tocopherol per gram model system was evaluated. Gas chromatography was used to monitor the concentration of methyl linoleate during storage while a respirometer was used to monitor oxygen uptake.

A typical chromatogram obtained from the gas chromatographic analysis of the lipid extract from the model system prior to storage is shown in Figure 13. The peak eluting at 5.5 minutes was characteristic of methyl linoleate. Data presented in Figure 14 show a similar chromatogram of the lipid extract from a stored model system. The methyl linoleate concentration decreased as storage time increased. Several peaks appeared near the solvent front as storage time increased.

The decrease of methyl linoleate concentration as a function of storage time is depicted in Figure 15. Loss of methyl linoleate was described by zero order kinetics through the range of storage parameters used in this experiment. The zero order rate constants for methyl linoleate degradation in a model system at various a_ws, storage container oxygen contents, storage temperatures, and initial α-tocopherol concentrations are shown in Tables 12 and 13.

The effect of a_w on the storage stability of methyl linoleate is represented by data in Figure 15. Methyl linoleate appeared to degrade the slowest at a_w of 0.23. The rate of methyl linoleate oxidation increased as the a_w was decreased to 0.11. A similar increase in methyl linoleate degradation was observed as the a_w was increased to 0.42, but slowed as the a_w was increased to 0.67. The linear prediction equations for the zero order rate constants of methyl linoleate degradation

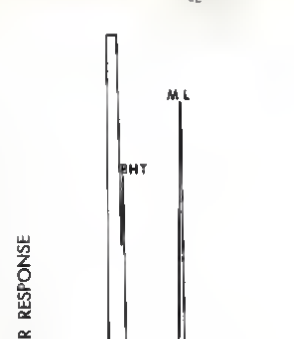


Figure 13: Typical gas chromatogram of the lipid extract from a model food system containing α-tocopherol and methyl linoleate prior to storage. BHT denotes butylated hydroxytoluene added during extraction and ML denotes methyl linolenate.

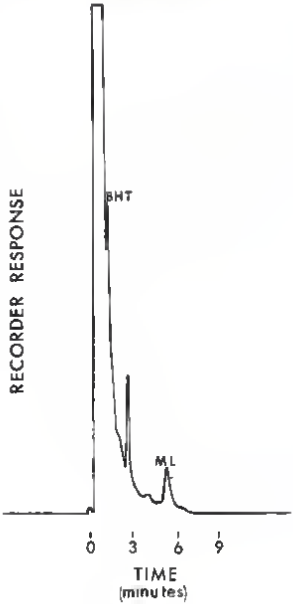


Figure 14. Typical gas chromatogram of the lipid extract from a model food system containing α-tocopherol and methyl linoleate after storage until approximately 90% of the initial methyl linoleate had degraded. BHT denotes butylated hydroxytoluene added during extraction and ML denotes methyl linoleate.

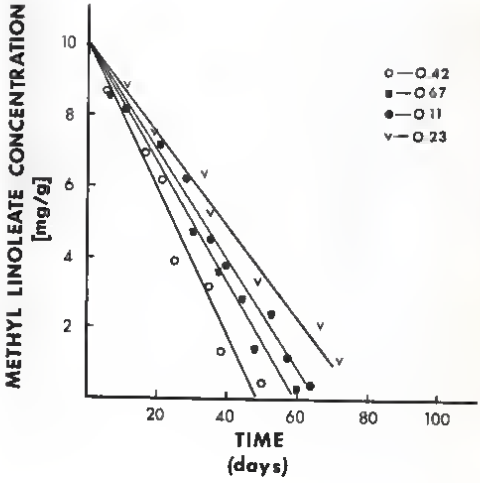


Figure 15 Zero order plot of methyl linoleate degradation in a dehydrated model food system containing 125 µg α-tocopherol per g stored in a 303 can (4.8 mmol gaseous O_2) at 30°C.

Table 12. Zero order rate constants[a] and prediction equations for methyl linoleate oxidation in a dehydrated model food system initially containing 125 μg α-tocopherol per g model system stored at various water activities, storage temperatures, and storage container oxygen contents.

Water Activity	Storage Temperature (°C) 37 TDT[b] Can	37 303[c] Can	30 TDT[b] Can	30 303[c] Can	20 TDT[b] Can	20 303[c] Can
0.87	3.32	1.94	2.13	1.88	0.84	0.71
0.42	4.11	2.04	2.32	2.02	0.99	0.82
0.23	2.37	1.66	1.45	1.35	0.58	0.42
0.11	3.71	2.78	1.84	1.55	0.81	0.55

Prediction equations (and standard error of coefficient estimates):

$$k^{a}_{TDT} = -2.51 + 0.640\ a_w + 0.148\ T \qquad R^2 = 0.84$$
$$(0.703)\quad (0.723)^{W}\quad (0.0219)$$

$$= 0.0372 - 30.5\ a_w + 0.148\ T + 98.4\ a_w^2 - 88.3\ a_w^3 \qquad R^2 = 0.92$$
$$(1.19)\quad (12.0)^{W}\quad (0.017)\quad (38.2)^{W}\quad (31.1)^{W}$$

$$k^{a}_{303} = -1.10 + 0.0218\ a_w + 0.0789\ T \qquad R^2 = 0.81$$
$$(0.453)\quad (0.488)^{W}\quad (0.0141)$$

$$= 0.583 - 19.7\ a_w + 0.0878\ T + 59.7\ a_w^2 - 50.9\ a_w^3 \qquad R^2 = 0.89$$
$$(0.833)\quad (8.45)^{W}\quad (0.0120)\quad (25.5)^{W}\quad (21.9)^{W}$$

[a] Zero order rate constant, x 10^{-1} mg methyl linoleate day^{-1}
[b] Contained 0.05 mmol gaseous O_2
[c] Contained 4.8 mmol gaseous O_2

Table 13. Zero order rate constants[a] and prediction equations for methyl linoleate oxidation in a dehydrated model food system initially containing 250 μg α-tocopherol per g model system stored at various water activities, storage temperatures, and storage container oxygen contents.

	Storage Temperature (°C)					
	37		30		20	
Water Activity	TDT[b] Cau	303[c] Cau	TDT[b] Cau	303[c] Cau	TDT[b] Cau	303[c] Cau
0.67	2.71	1.66	1.90	1.10	0.66	0.50
0.62	4.01	1.96	2.10	1.91	0.87	0.66
0.23	2.16	1.60	1.21	1.06	0.55	0.38
0.11	3.61	2.41	1.43	1.15	0.66	0.43

Prediction equations (and standard error of coefficient estimates):

$$\hat{k}^{a}_{TDT} = \underset{(0.79)}{-2.36} + \underset{(0.811)}{0.321}\, a_w + \underset{(0.0246)}{0.140}\, T \qquad R^2 = 0.78$$

$$= \underset{(1.39)}{0.180} - \underset{(14.1)}{31.4}\, a_w + \underset{(0.0201)}{0.140}\, T + \underset{(42.6)}{102}\, a_w^2 - \underset{(36.6)}{90.4}\, a_w^3 \qquad R^2 = 0.88$$

$$\hat{k}^{a}_{303} = \underset{(0.428)}{-1.13} - \underset{(0.440)}{0.136}\, a_w + \underset{(0.0133)}{0.083}\, T \qquad R^2 = 0.81$$

$$= \underset{(0.755)}{0.146} - \underset{(7.65)}{16.4}\, a_w + \underset{(0.0105)}{0.083}\, T + \underset{(23.1)}{53.2}\, a_w^2 - \underset{(19.8)}{47.7}\, a_w^3 \qquad R^2 = 0.90$$

[a] Zero order rate constants, $\times 10^{-1}$ mg methyl linoleate day^{-1}
[b] Contained 0.05 mmol gaseous O_2
[c] Contained 4.8 mmol gaseous O_2

(Tables 12 and 13) indicate that the rate constants are not significantly affected by a_w. The cubic prediction equations with respect to a_w have larger R^2 values than do the linear prediction equations. Paired difference t-tests of the rate constants (independent of temperature, storage container oxygen content, or initial α-tocopherol concentration) showed the average value of the rate constants at a_w 0.42 was significantly greater than the average value of the rate constant at a_w 0.67 ($p \leq 0.01$), a_w 0.23 ($p \leq 0.01$), and 0.11 ($p \leq 0.05$). Similarly, the average values of the rate constants at a_w 0.67 and 0.11 were not significantly different ($p \leq 0.01$), but each was significantly greater ($p \leq 0.01$) than the average from a_w 0.23.

Methyl linoleate destruction was affected by the content of oxygen in the storage container. The rate of methyl linoleate oxidation was significantly greater ($p \leq 0.01$) when the model system was stored with 0.05 mmole O_2 rather than with 4.8 mmol O_2. No deviation from the zero order degradation pattern was observed indicating that the oxygen content in either storage container did not limit the rate of methyl linoleate degradation.

The concentration of α-tocopherol in the model system did affect the observed rate of methyl linolenate degradation. The rate of methyl linoleate loss was significantly lower ($p \leq 0.01$) when the initial concentration of α-tocopherol was higher.

A significant temperature dependence of methyl linoleate oxidation was described by the prediction equations (Tables 12 and 13). The apparent activation energies (Ea) were calculated and are shown in Table 14. The Ea's ranged from 11.1 to 18.2 kcal mol^{-1} and no Ea was significantly different from the others at $p < 0.01$ level.

Table 14. Apparent activation energies[a] for methyl linolenate oxidation in a dehydrated model food system stored at various water activities, storage container oxygen contents, and initial α-tocopherol concentrations.

	α-Tocopherol Concentration			
	125 μg α-tocopherol per g		250 μg α-tocopherol per g	
Water Activity	TDT[b] Can	303[c] Can	TDT[b] Can	303[c] Can
0.67	14.6	15.2	15.3	12.8
0.42	16.2	13.0	16.2	12.1
0.23	14.8	11.1	14.5	15.5
0.11	15.7	16.8	17.7	18.2

[a] Apparent activation energies, kcal mol^{-1}
[b] Contained 0.05 mmol gaseous O_2
[c] Contained 4.8 mmol gaseous O_2

A typical oxygen uptake pattern of the samples containing methyl linoleate and various quantities of α-tocopherol is shown by the data in Figure 16. Samples containing α-tocopherol and methyl linoleate showed an induction period before oxygen uptake was observed. The oxygen uptake occurred with an initial linear uptake followed by an asymptotical approach to a limiting value.

The rates of oxygen uptake and the induction periods prior to oxygen uptake by the model system at various a_ws and α-tocopherol concentrations are shown in Table 15. The oxygen uptake rates were the most rapid at a_w of 0.42 and the slowest at a_w of 0.23, but were not linearly effected by a_w. The inductino periods ranged from 1.2 to 4.6 days and did not follow a linear pattern as a function of a_w.

Metal Concentration of Model System

The concentration of total iron and copper in the model system containing no fat was determined by atomic absorbance spectrophotometry. The model system contained 17.39 and 2.17 ppm of iron and copper, respectively. This represents 311.3 nmol of total iron and 34.1 nmol of total copper per gram model system.

Bioassay of α-Tocopherol After Storage

The biological activity of α-tocopherol stored in dehydrated model food systems containing no fat, saturated fat, and unsaturated lipids was determined. The biological activity of α-tocopheryl amide, α-tocopheryl quinone, and α-tocopheryl dimer was also determined.

Blood plasma from each rat was analyzed for aspartate aminotransferase (AspAT) and pyruvate kinase (PK) activities. Plasma α-tocopherol concentration was also determined for each rat. Plasma AspAT activity was found to be unaffected by the dose of α-tocopherol ingested by the

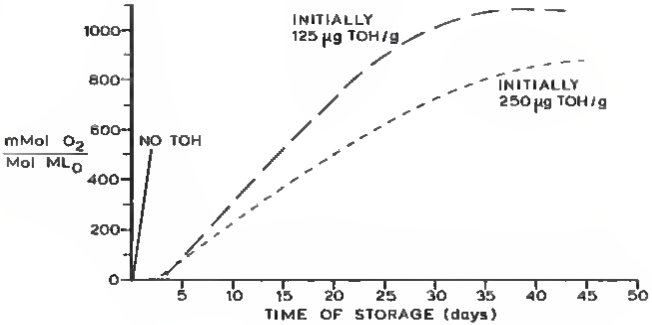


Figure 16. Oxygen uptake by a dehydrated model food system containing methyl linolenate stored at 37°C, water activity of 0.11

Table 15. Rate of oxygen uptake[a] and induction period[b] of dehydrated model food system containing 1% methyl linoleate stored at 37°C at various water activities and initial α-tocopherol concentrations.

	Initial α-Tocopherol Concentration			
	125 μg per g		250 μg per g	
Water Activity	Rate of O_2 Uptake[a]	Induction Period[b]	Rate of O_2 Uptake[a]	Induction Period[b]
0.67	28.6	1.2	25.3	4.6
0.42	36.3	4.1	30.3	3.2
0.23	25.8	4.2	20.7	4.2
0.11	34.3	3.2	29.7	2.9

[a] Rate of O_2 uptake, (mmol O_2) x (mol methyl linoleate^{-1}) x (day^{-1})
[b] Induction period, days

rate, so this assay was not used in the quantitation of α-tocopherol biological activity.

Plasma PK activity and α-tocopherol concentration were dependent upon the amount of α-tocopherol ingested (Figures 17 and 18). An increase in the amount of α-tocopherol ingested caused a decrease in the PK activity and an increase in the plasma α-tocopherol concentration. The dietary dose dependency ranged from 0 to 2.5 mg α-tocopherol per kg body weight-day for plasma α-tocopherol concentration, and from 0.08 to 1.6 mg α-tocopherol per kg body weight-day for PK activity.

The biological activity of α-tocopherol stored in a dehydrated model food system was determined by comparing the response of plasma α-tocopherol concentration and PK activity to the response curves from feeding freshly prepared model system containing graded amounts of α-tocopherol. These data, and the corresponding HPLC determined α-tocopherol concentrations, are shown in Table 16. Data obtained in a similar manner for α-tocopheryl acide, α-tocopheryl quinone, and tocopheryl dimer are shown in Table 17. The reported mean α-tocopherol equivalents represent the equivalent amount of α-tocopherol required to cause a response equal to the group mean response from the model system. The reported ranges represent the equivalent α-tocopherol concentration required to cause a response of + or - one standard error from the mean group response. Since the standard response curve is curvilinear, the reported mean α-tocopherol equivalents are not the same as the mean of the ranges.

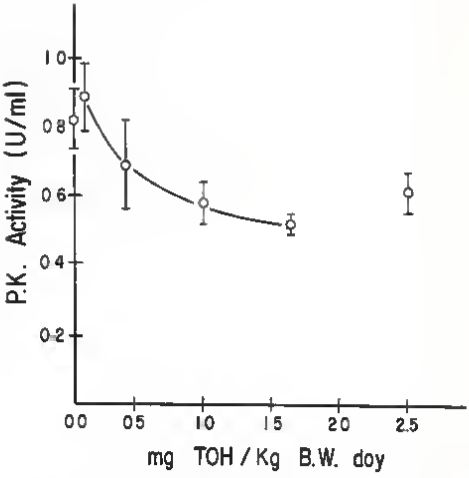


Figure 17 Plasma pyruvate kinase activity as a function of amount of α-tocopherol fed during the 4 day vitamin E repletion period. Bars represent ± one standard error of mean group response.

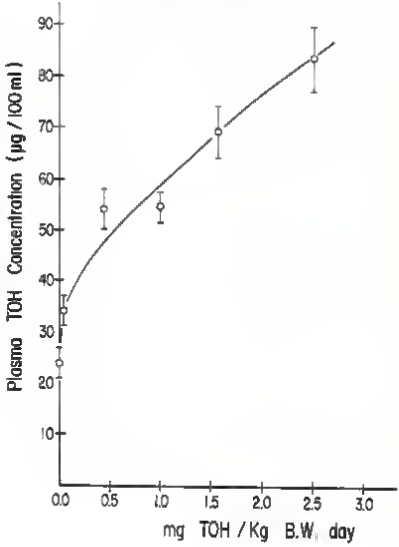


Figure 18 Plasma α-tocopherol concentration response as a function of α-tocopherol consumed during the 4 day vitamin E repletion period. Bars represent ± one standard error of mean group response.

Table 15. The concentration of α-tocopherol in model feed systems before and after storage, and the biological α-tocopherol equivalents as determined by plasma α-tocopherol concentration and pyruvate kinase activity in a rat bioassay.

Model System	Initial α-tocopherol concentration[c] (μg/g)	Final α-tocopherol concentration[c] (μg/g)	Mean (and range)[d] α-tocopherol equivalents determined by: Plasma α-tocopherol (μg/g)	Pyruvate kinase (μg/g)
No fat	195.5	61.7	>291.3	87.4 (45.4-174.8)
Saturated fat[a]	132.2	36.7	53.2 (21.3-100.4)	19.5 (0-53.2)
Unsaturated fat[b]	125.2	11.6	<3.0 (0-9.6)	<9.6 (0-9.6)

[a] Contained 1% hydrogenated coconut fat
[b] Contained 1% methyl linoleate
[c] Determined by high performance liquid chromatography procedure
[d] Range of α-tocopherol equivalents of plus or minus one standard error of response

Table 17. The fortification level and α-tocopherol biological activity equivalents of β-tocopheryl oxide, β-tocopheryl quinone, and α-tocopheryl dimer.

Degradation Product	Concentration in diet (μg/g)	Mean (and range)[a] α-tocopherol equivalents determined by: Plasma α-tocopherol (μg/g)	Pyruvate kinase (μg/g)
α-tocopheryl oxide	412.5	0 (0–28.0)	524.3 (279.6–559.2)
β-tocopheryl quinone	412.5	0 (0–29.3)	143.0 (77.3–296.9)
α-tocopheryl dimer	412.5	36.4 (29.1–72.8)	29.1 (0–109.1)

[a]Range of α-tocopherol equivalents of plus or minus one standard error or response

DISCUSSION

The storage stability of α-tocopherol in a dehydrated model food system containing no lipid, saturated lipids, or unsaturated lipids was studied. The storage parameters evaluated included water activity (a_w), lipid composition, storage temperature, and the oxygen content in the storage container. The biological activity of α-tocopherol after storage and of α-tocopheryl oxide, α-tocopheryl quinone, and α-tocopheryl dimer was also evaluated.

α-Tocopherol Stability. No Fat Model System

The concentration of α-tocopherol in a dehydrated model food system containing no fat was monitored as a function of storage time. The model system was stored at a_w of 0.10, 0.24, 0.42, or 0.65 at 20, 30, or 37°C with molar ratios of oxygen to α-tocopherol of 15:1 (TDT can containing 0.05 mmol oxygen) or 1450:1 (303 can containing 4.8 mmol oxygen). Figures 5 and 7 show high performance liquid chromatograms of the lipid extract of a α-tocopherol fortified dehydrated model food system containing no added fat. A typical chromatogram of the lipid extract of the model food system obtained at zero storage time is shown in Figure 6. Figure 7 shows a chromatogram obtained after storage of the model food system until approximately 75% of the initial α-tocopherol had degraded. The concentration of α-tocopherol decreased during storage of the model food system containing no fat. Peaks characteristic of two known degradation products of α-tocopherol were detected after storage of the model food system containing no fat (Figure 7). Although not

quantitated because of their low concentration and absorptivity, these peaks are believed to be α-tocopheryl oxide and α-tocopheryl quinone. The HPLC retention characteristics and the absorptivities of these peaks are similar to those for α-tocopheryl oxide and α-tocopheryl quinone, which supports the tentative identification of these compounds. Further analysis of these peaks was not done.

The decrease of α-tocopherol concentration in the model food system was monitored as a function of storage time. Alpha-tocopherol degradation in the model food system containing no lipid was modeled by first order kinetics (Figure 4). This pattern of degradation was observed at all a_ws, temperatures, and the two molar ratios of oxygen to α-tocopherol studied.

A_w Effect

Salwin (1963) hypothesized that nutrients should exhibit maximum stability when a food product is stored at its B.E.T. monomolecular moisture content. The B.E.T. monomolecular moisture content for the model food system used in the present study corresponds to an a_w of 0.24 (Bach, 1974). The rate of α-tocopherol degradation during storage in the model system decreased as the a_w was decreased from 0.65 to 0.10. These data indicate that α-tocopherol in a food system containing no fat is most stable at a a_w below the B.E.T. monomolecular moisture content.

The degradation rate of α-tocopherol increased as a function of a_w through the range of 0.10 to 0.65 (Table 5). This relationship between a_w and degradation rates is characteristic of the degradation of water soluble nutrients (Figure 3). Because α-tocopherol is not water soluble, these data indicate that a reactant which is water soluble is required for degradation of α-tocopherol. The reactivity of the water

soluble reactant and its availability for reaction with α-tocopherol would be expected to affect the degradation rate of α-tocopherol.

The availability of α-tocopherol for reaction with these water soluble reactants is influenced by the physical state of the α-tocopherol in the model system. Dispersion of α-tocopherol in the model systems used in these stability studies was accomplished by homogenization of a 40% solids slurry prior to dehydration. Voth and Miller (1958) reported that in an aqueous media with a molar ratio of α-tocopherol to protein of 6:1, the α-tocopherol was bound to proteins. Voth and Miller (1958) theorized that α-tocopherol bound to the negatively charged amino acids in proteins. Since α-tocopherol is hydrophobic, α-tocopherol would be expected to bind to the hydrophobic portions of proteins and not to the polar, negatively charged amino acids. In the model system slurry, α-tocopherol was homogeneously dispersed, and based on protein to α-tocopherol ratios reported by Voth and Miller (1958), the finely dispersed hydrophobic α-tocopherol molecules would be expected to be bound to proteins in the model system and be uniformly distributed. Removal of water from the model system slurry by freeze drying would not affect the dispersion of α-tocopherol throughout the model food system matrix. Thus, reactants involved in the degradation of α-tocopherol would need to diffuse through the model system matrix and its associated water layer(s) to react with α-tocopherol.

Labuza (1976) reported that an increase in the a_w or moisture content of a food influences the availability and reactivity of water soluble reactants. The increased a_w was reported to cause swelling of bound surfaces, dissolution of precipitated crystals, and lowering of viscosity of the aqueous phase. These parameters were associated with an

increased solubility of reactants or increased rate of migration of water soluble reactants, resulting in an increased rate of water soluble nutrient loss. Similarly, as the a_w of the model system used in these storage studies was increased, the model system matrix surrounding the α-tocopherol would become hydrated, and the reactivity and the rate of migration of water soluble reactants would increase. Data in Table 5 show the rate of α-tocopherol degradation increased as the a_w of the model food system increased from 0.10 to 0.65, indicating an increase in availability and reactivity of water soluble reactants.

Two previously discussed mechanisms of α-tocopherol degradation could occur in the model food system containing no fat (Kofler et al., 1972 and Cort et al., 1978). Both of these mechanisms involve water soluble reactants. Although the water soluble compounds which react with α-tocopherol were not identified, they are theorized to include metal ions and oxygen.

Metal Ion Effect

Cort et al. (1978) reported that only the oxidized forms of transition metals such as iron and copper react with α-tocopherol. Analysis of the model system used in the present experiment confirmed the presence of 311.3 nmol iron, 34.1 nmol copper, and 290 nmol of α-tocopherol per g of model system. Iron is known to be readily oxidized to the ferric form in the presence of oxygen and water. The probability that iron or copper would be in their oxidized states would be enhanced by an increase of the moisture content or oxygen solubility of the model system. Chou and Labuza (1974) reported that at low metal concentrations (10-50 ppm) an increase in moisture content caused a faster rate of lipid oxidation due to lowered viscosity of the aqueous phase, higher

solubility of the reactants, and swelling of the surfaces to increase the number of catalytic sites. In the model system used in the present study which contained low levels of metal, the diffusion rate of the metal ions would be expected to increase as the a_w is increased. Thus, the rate of α-tocopherol degradation in the model food system may be a function of the rate of metal ion oxidation or the rate of migration of the metal ion to α-tocopherol. These data indicate that metal ions may influence the stability of α-tocopherol. Removal of metal ions from a food product would be expected to enhance the stability of α-tocopherol.

Oxygen Effect

Kofler et al. (1972) reported that oxygen is required for the degradation of α-tocopherol and a limited oxygen content would be expected to decrease the rate of α-tocopherol degradation. Halton and Fisher (1937) and Kirk (1978) theorized that the a_w may control the oxygen solubility and the rate of oxygen diffusion in the aqueous medium of foods. Recently, Mohr (1980) proposed that the mass transfer of oxygen affects the rate of oxidation of ascorbic acid in dehydrated foods. In the model food system used in this study, oxygen solubility and diffusion would be affected by a_w. The soluble solids concentration and the bound water in the model system matrix would be expected to control the availability of oxygen for α-tocopherol degradation. As the a_w is increased, the amount of dissolved oxygen present and the rate of migration of that dissolved oxygen would increase, resulting in an increased degradative rate of α-tocopherol.

The amount of soluble oxygen required for oxidative of α-tocopherol was calculated. A molecule of α-tocopherol contains two oxygen atoms while α-tocopheryl oxide or α-tocopheryl quinone contains three atoms of

oxygen, indicating that a minimum of 0.5 mol O_2 is required for conversion of one mol α-tocopherol to α-tocopheryl oxide or α-tocopheryl quinone. The maximum solubility of oxygen dissolved in the moisture in the model food system was calculated assuming the model system contained 10% water (a_w of 0.65 at 37°C) and using a high estimate of the solubility of oxygen in water (4.89 cm^3 O_2 per 100 cm^3 H_2O at 0°C). This value, even though it is a high estimate of oxygen solubility in the model food system, was 4.58 X 10^{-8} mol oxygen per g model system. This resulted in a molar ratio of dissolved oxygen to α-tocopherol of 0.16:1 since the model system was fortified at a level of 2.9 X 10^{-7} mol α-tocopherol per g. This molar ratio is smaller than the ratio of oxygen required for oxidation of α-tocopherol (0.5:1) indicating that all of the α-tocopherol in the model system cannot be oxidized by the soluble oxygen in the aqueous phase of the model food system. Thus, dissolution of gaseous oxygen into the moisture of the model system was required for oxidation of α-tocopherol.

The dissolution of gaseous oxygen into the moisture in the model system is controlled by the equilibrium constant:

$$K = \frac{[O_2]_d}{[O_2]_g}$$

where $[O_2]_d$ and $[O_2]_g$ denotes the concentration of dissolved and gaseous oxygen, respectively. As dissolved oxygen was removed from the moisture of the model system by reaction with α-tocopherol, gaseous oxygen would be transported into the aqueous phase of the model system. Removal of oxygen from the headspace of the container would reduce the partial pressure of oxygen in the container. A lowered partial pressure of oxygen would decrease the driving force for mass transfer and dissolution of oxygen into the aqueous medium of the model system. This change of

the partial pressure of oxygen would be most pronounced when the initial concentration of gaseous oxygen is lower. As the partial pressure of gaseous oxygen is decreased, the concentration of dissolved oxygen would be decreased according to the equilibrium equation. This decreased concentration of dissolved oxygen would be expected to decrease the rate of α-tocopherol degradation.

The rate of α-tocopherol degradation in the model system containing an lipid was affected by the oxygen content in the storage containers (Table 5 and Figure 12). The molar ratios of gaseous oxygen to α-tocopherol present in the storage containers were calculated to be approximately 15:1 (TDT can) or 1450:1 (303 can). Adequate oxygen was present in both types of storage containers to degrade all of the α-tocopherol in the model food system. However, the rate of α-tocopherol degradation was greater ($p \leq 0.05$) when the samples were stored with the larger ratio of gaseous oxygen to α-tocopherol.

The affect of the molar ratio of gaseous oxygen to α-tocopherol was most pronounced at higher a_w's and higher storage temperatures, where the rate of α-tocopherol degradation approached its maximum. Although the solubility of oxygen is decreased as the temperature is increased, an increased temperature will increase the mass transfer of oxygen into the aqueous phase of the model system and decrease the viscosity of the aqueous phase. In addition, oxygen solubility will increase as the a_w is increased. The diffusion rate of oxygen is increased when both the temperature and a_w are increased. The effect of the molar ratio of gaseous oxygen to α-tocopherol was most pronounced when the rate of α-tocopherol degradation was rapid (at 37°C) and the diffusion of oxygen approached a maximum (at a_w of 0.65). This indicates that the effect of

gaseous oxygen content on the stability of α-tocopherol in a food system containing no fat would occur only when the dissolved oxygen can readily diffuse through the model system to react with α-tocopherol.

Activating Parameters

Apparent activation energies (Ea) for the degradation of α-tocopherol during storage in the model food system containing no fat are shown in Table 6. The Ea's ranged from 8.9 to 13.1 Kcal mol^{-1}. No significant differences ($p \leq 0.01$) were found to exist among any of the Ea's. These data indicate that the energy required to form the activated α-tocopherol complex was not effected by the a_w or oxygen concentration in the storage container.

The thermodynamic activation parameters including enthalpy ($\Delta H^{\ddagger}$), entropy ($\Delta S^{\ddagger}$), and Gibbs free energy ($\Delta G^{\ddagger}$) of activation are summarized in Table 7. Water activity did not influence $\Delta H^{\ddagger}$ or $\Delta S^{\ddagger}$ in a linear manner. The $\Delta G^{\ddagger}$ ranged from 27.1 to 27.4 throughout the range of a_w and oxygen contents used in this experiment, but the $\Delta G^{\ddagger}$ were not significantly different ($p \leq 0.05$). This isokinetic relationship is theorized to be exhibited only by reactions in which solvent changes do not result in a change of the reaction mechanism (Leffler, 1955). The fact that the degradation pattern of α-tocopherol in the model system adhered to the isokinetic relationship indicates that the reaction mechanism of α-tocopherol degradation was not altered by a change in the a_w.

α-Tocopherol Stability: Saturated Fat Model System

The concentration of α-tocopherol in a dehydrated model food system containing 12 hydrogenated coconut fat was monitored as a function of storage time. The model system was stored with a molar ratio of oxygen to α-tocopherol of 1450:1 (303 cans) at a_w of 0.11, 0.23, 0.42, or 0.67

at 37°C. The degradation of α-tocopherol in a dehydrated model food system containing 1% hydrogenated coconut fat was described by first order kinetics (Figure 8). These data represent a non-lipid mediated degradation of α-tocopherol in a model food system containing lipids.

The first order rate constants for α-tocopherol degradation in a model food system containing saturated fat (Table 8) were not significantly different ($p<0.01$) from the corresponding rate constants for α-tocopherol loss in model systems containing no fat (Table 5) stored under the same conditions. The rates of α-tocopherol degradation in the model systems containing no fat or saturated lipids were a function of a_w. In both cases, the α-tocopherol degradation rate was highest at the higher a_ws and decreased as the a_w was decreased (Table 8). Thus, it again appears that the α-tocopherol is reacting with a water soluble reactant and that the activity, solubility, or mobility of these reactants is increased as the a_w is increased as discussed in the previous section.

From these data, it appears that the storage stability of α-tocopherol is similar whether the food system contains no fat or a saturated fat. Because saturated fats have been shown not to oxidize at appreciable rates in the model food system (Widicus and Kirk, 1980), the storage stability of α-tocopherol in the model food system containing a saturated fat would represent a degradation of α-tocopherol which was not mediated by autoxidation of unsaturated lipids. Variations of the storage parameters including a_w, oxygen content in the storage container, and temperature would be expected to cause similar effects on the degradation rate of α-tocopherol regardless of whether the model system contained no fat or saturated lipids.

α-Tocopherol Stability. Unsaturated Fat Model System

The third portion of this study concerns the stability of α-tocopherol in a dehydrated model food system containing an unsaturated fat, methyl linoleate. The storage parameters which were varied in this third study included: a_w (0.11, 0.23, 0.42, and 0.67), temperature (20, 30, and 37°C), storage container oxygen content (0.05 or 4.8 cmml oxygen per container), and the initial α-tocopherol concentration (125 or 250 μg α-tocopherol per g model system).

The concentration of α-tocopherol in the model system during storage was monitored by a high performance liquid chromatographic (HPLC) procedure. Figure 9 represent a HPLC chromatogram of the lipid extract of the model system containing α-tocopherol and methyl linoleate before storage. Figure 10 is a chromatogram of the extract from the same model system following storage until approximately 87.5% of the initial α-tocopherol had degraded. Comparison of the chromatograms in Figures 9 and 10 demonstrates that the concentration of both methyl linoleate and α-tocopherol decreased with storage time (Table 9, 10, 12, and 13).

Several peaks other than those characteristic of methyl linoleate and α-tocopherol were detected after storage of the model system. Peaks characteristic of α-tocopheryl oxide (TOX) and α-tocopheryl quinone (TQ) were among the new peaks. Alpha-tocopheryl oxide and α-tocopheryl quinone were also detected in the model systems containing no fat following storage. These data indicate that the same degradation products of α-tocopherol were formed regardless of the presence or type of lipid in the model system. Two other large peaks eluting at 10.5 and 12 minutes were not identified, but the size of these peaks increased as a function of storage time. Peaks with similar retention times to those of the two

unknown peaks were detected when undistilled chloroform was chromatographed under identical conditions. These peaks were not detected when the extraction was done with methanol or hexane. Since chloroform is known to be unstable, it is probable that the two peaks eluting in 10.5 and 12 minutes were degradation products produced from reaction of chloroform with reactants or oxidation products formed during storage of the model systems.

The percent recovery of a known amount of α-tocopherol added during the chloroform:ethanol:water extraction of the lipids from the model system containing unsaturated lipids decreased as a function of the length of storage of the model system. This indicates that compounds were formed during storage of the model system containing methyl linoleate which oxidized α-tocopherol during extraction. Addition of 0.002 g BHT to the extraction solutions prevented this loss of α-tocopherol during extraction. The addition of BHT to the extraction solutions was done throughout these storage stability studies, and the extraction recoveries of α-tocopherol were consistently 90%. This indicates that α-tocopherol, in the presence of BHT, was not being oxidized during extraction, and that oxidation products formed during storage of the model system did not affect the extraction of α-tocopherol.

The degradation of α-tocopherol in this model food system containing an unsaturated lipid, methyl linoleate, was described by zero order kinetics (Figure 11). Deviations from zero order kinetic plots were not observed with variations of the a_w from 0.11 to 0.67, the storage temperature from 20 to 37°C, or the initial concentration of α-tocopherol from 125 to 250 μg α-tocopherol per g model system. The loss of α-tocopherol adhered to zero order kinetics when the model system was stored in con-

taining with 4.8 meal O_2, but did not follow the zero kinetic model when stored with 0.05 meal O_2.

As previously discussed, the identifying feature of a zero order reaction is that the degradation rate is independent of the initial concentration of the reactant. Even though the degradation of α-tocopherol fit zero order kinetic plots, the reaction mechanism was not zero order with respect to α-tocopherol since the rate of α-tocopherol loss was accelerated as the initial concentration of α-tocopherol was increased (Tables 9 and 10). These data indicate that the rate of α-tocopherol degradation in a food system containing unsaturated fat is controlled by the concentration of a reactant other than α-tocopherol. The degradation reaction of α-tocopherol in this model system must be more complex than simple zero order kinetic theory can explain. This complex reaction will be discussed further in the section dealing with the stability of methyl linolenate.

A_w Effect

The rate of α-tocopherol degradation in a model system containing methyl linolenate was affected by a_w (Figure 11). The degradation rate was slowest at a_w of 0.23, a value near the B.E.T. monomolecular moisture content (a_w of 0.24) (Bach, 1974). The rate of α-tocopherol loss in the model system containing methyl linolenate was increased as the a_w was lowered to 0.11 or raised to 0.42 or 0.67 (Tables 9 and 10 and Figure 11). The degradation of α-tocopherol in a model system containing methyl linolenate supports the hypothesis of Salwin (1963) which states that autrleats are the most stable in foods stored at their B.E.T. monomolecular moisture content

The affect of a_w on the storage stability of α-tocopherol in the model system containing methyl linoleate is similar to the effect of a_w on the stability of unsaturated lipids, as summarized by Labuza (1968) (Figure 3). These data indicate that α-tocopherol degraded by a mechanism that is dependent on, or similar to, the oxidation of monounsaturated lipids.

Metal Ions Effect

The presence of metal ions in the model food system affects the rate of oxidation of α-tocopherol. Cort et al. (1978) reported that metal ions in their higher valence states react with α-tocopherol to cause oxidation of α-tocopherol. Gruger and Teppel (1970 a,b) reported that decomposition of lipid hydroperoxides at low concentrations occurred as:

$$ROOH + Fe^{+2} \longrightarrow RO\cdot + OH^{-} + Fe^{+3},$$

indicating that the rate of iron oxidation is affected by the decomposition of methyl linoleate hydroperoxides. In the model system containing methyl linoleate, the rate of metal ion oxidation to its higher valence state would be affected by moisture, oxygen, and methyl linoleate hydroperoxide breakdown. Thus, the rate of α-tocopherol degradation in the model system containing methyl linoleate is probably dependent upon the rate of metal ion oxidation.

Lipid Oxidation Effect

In addition to the effect of metal ions, the oxidation of methyl linoleate yields reactants which oxidize α-tocopherol. Gruger and Teppel (1970 a,b) reported that the reaction of free radicals produced from lipid oxidation results in the oxidation of α-tocopherol and termination of the free radicals. The oxidation of methyl linoleate in the model

system during storage in conditions previously discussed would be expected to form free radicals which react with oxidizable substrates. Labuza (1980 a) theorized that changes in a_w of a food product may affect the hydrogen bonding of peroxides or the rate of free radical reaction with non-lipid species, such as proteins. The affect of increasing the a_w on hydrogen bonding or protein oxidation would be expected to cause a change in the reactivity of free radicals with α-tocopherol in the model food system.

Oxygen Effect

In the present study, the oxygen content in the storage container appeared to affect the rate of α-tocopherol degradation in the model food system containing methyl linoleate. The rate of α-tocopherol loss during storage in the TDT can was greater than the rate of α-tocopherol lose during storage in the 303 can. This result is the opposite of the effect of oxygen content on α-tocopherol stability in a model system containing no fat. As discussed previously, the mass transfer of gaseous oxygen into the aqueous phase of the model system is favored when the concentration of oxygen in the container is higher. The degradation rate of α-tocopherol is affected by the amount of dissolved oxygen, thus the model system in the 303 can should exhibit a greater rate of α-tocopherol degradation than the model system in the TDT can. This effect of container oxygen content was not observed. Therefore, the rate of α-tocopherol degradation in a model system containing methyl linoleate does not appear to be controlled by rate of oxygen dissolution.

This unexpected effect of container oxygen content is probably due to the fact that oxygen is more soluble in unsaturated lipids than in water (Ke and Ackman, 1973). The oxygen required for oxidation of α-

tocopherol may be present in the lipid fraction and thus mask any effect of the storage container oxygen content. Furthermore, the fact that the oxygen-binding methyl linoleate was mixed with α-tocopherol prior to blending with the model system probably ensured the presence of sufficient amounts of dissolved oxygen for α-tocopherol oxidation, regardless of the oxygen content of the storage container.

The concentration and composition of the degradation products formed from methyl linoleate oxidation probably influenced the degradation of α-tocopherol stored in TDT or 303 cans. During the degradation of methyl linoleate, many free radicals, peroxides, and hydroperoxides are produced (Dugan, 1976). The concentration and composition of the methyl linoleate degradation products formed during storage may differ when the model food system is stored with 0.05 mmol oxygen (TDT can) or with 4.8 mmol oxygen (303 can). Different concentrations and/or composition of methyl linoleate degradation products would react differently with α-tocopherol. Thus, the observed degradation as a function of storage container may be due to the different concentration and/or composition of the methyl linoleate degradation products. Further discussion of the production of degradation products formed during storage of methyl linoleate is presented in the discussion of methyl linoleate stability.

Another possible factor in the deviation from the anticipated degradation rate in the TDT cans was the greater concentration of volatile degradation products due to the limited headspace volume. Many volatile degradation products are formed during degradation of unsaturated lipids (Dugan, 1976). These volatile free radicals or other reactants would cause oxidation of α-tocopherol. The concentration of these volatile free radicals or reactants is higher in the TDT can since the total vol-

one of gas is approximately 100 times less than the gaseous volume in the 303 cans. This increased concentration of volatile reactants would affect the volatile reactant dissolution equilibrium

$$K = \frac{[\text{Volatile Reactant}]_{\text{dissolved}}}{[\text{Volatile Reactant}]_{\text{gaseous}}}$$

An increased concentration of volatile reactants in the gaseous headspace results in an increased concentration of dissolved volatile reactants, which catalyze the degradation of α-tocopherol. Further discussion of the production of volatile products produced during lipid oxidation is presented in the discussion of methyl linoleate stability.

A deviation from zero order kinetics for α-tocopherol destruction was noted during the storage study involving two model systems containing α-tocopherol and methyl linoleate in TDT cans (Figure 12). α-Tocopherol degradation in model systems containing either 125 or 250 µg α-tocopherol per g model system was negligible when the concentration of α-tocopherol reached a level of 20 µg of α-tocopherol per g model food system. These data indicate that the primary reactant required for degradation of α-tocopherol was depleted during storage of the model system in TDT cans. It is proposed that this limiting reactant was oxygen since oxygen is the only known reactant which is affected by storage of the model system in TDT or 303 cans.

The oxygen required for α-tocopherol degradation was also consumed by the oxidation of methyl linoleate. One of the factors affecting the rate of methyl linoleate oxidation is the concentration of α-tocopherol in the model system. When the concentration of α-tocopherol was 125 µg per g, the consumption of oxygen by methyl linoleate was greater than the consumption of oxygen by methyl linoleate when the initial

α-tocopherol concentration was 250 μg per g (Figure 16). This faster rate of methyl linoleate degradation when the initial concentration of α-tocopherol was lower resulted in a more rapid consumption of the available oxygen. Thus, α-tocopherol had insufficient oxygen available for degradation below a level of 20 μg per g, regardless of the initial concentration of α-tocopherol.

Activation Parameters

The degradation of α-tocopherol was influenced by storage temperature. The temperature dependence of α-tocopherol degradation in a model food system containing methyl linoleate could be described by the Arrhenius equation. The apparent activation energies (Ea) reported in Table 11 ranged from 6.0 to 13.4 Kcal mol^{-1}. The values of Ea are not significantly different ($p \leq 0.05$). It does not appear that the Ea's were a function of initial α-tocopherol concentration or oxygen content in the container. Water activity appeared to have an effect on the Ea's. The Ea's were generally lowest at 0.42 a_w and highest at 0.67 a_w. Labuza (1980 b) reviewed the literature dealing with Ea's for reactions as a function of a_w and concluded that, in general, Ea's for nutrients in food products are not completely effected by a_w. Although an apparent trend in the Ea for α-tocopherol destruction as a function of a_w in the model systems was noted, the Ea's were not significantly different ($p \leq 0.05$), and this trend is not supported by the literature.

Methyl Linoleate Stability, Unsaturated Fat Model Systems

In addition to studying the stability of α-tocopherol in a model food system containing methyl linoleate, the stability of methyl linoleate was also studied. The simultaneous study of methyl linoleate and α-tocopherol stability in the same model food system provides information concerning the interaction of these two compounds.

The concentration of methyl linoleate was monitored by a gas liquid chromatography (GLC) procedure. BHT was added during extraction to prevent oxidation of methyl linoleate. Recoveries of methyl linoleate added during the extraction were ≥90% throughout the storage studies. Figure 13 depicts a chromatogram of the lipid extract from a model food system prior to storage. A GLC chromatogram of the lipid extract from a model food system stored until approximately 90% of the methyl linoleate had degraded is shown in Figure 14. Methyl linoleate degraded, and additional GLC peaks were detected during storage of the model food system.

Methyl linoleate degradation during storage of a dehydrated model food system containing α-tocopherol could be described by zero order kinetics (Figure 15). Deviations from zero order kinetic plots were not observed as a function of a_w, oxygen content in the container, α-tocopherol concentration, or storage temperature (Tables 12 and 13).

A_w Effect

Methyl linoleate was most stable at a_w of 0.23 (Figure 15), a value near the B.E.T. monomolecular moisture content for this model system (Bach, 1974). This observed stability pattern of an unsaturated lipid in a dehydrated food system has been reported by numerous investigators (Thompson et al., 1962; Martinez and Labuza, 1968; Labuza et al., 1969; Tuomy et al., 1969, Heidelbaugh et al., 1971; Labuza et al., 1972; and Quast and Karel, 1972).

The change in the rate of methyl linoleate oxidation as affected by the a_w was similar to the pattern described by Labuza (1976) (Figure 3). The rate of methyl linoleate oxidation increased as the a_w was increased within the multilayer region from 0.23 (the B.E.T. monomolecular moisture content) to 0.47. As previously discussed, the observed increase in the

rate of a reaction as the a_w is increased is due to swelling of bound surfaces to expose more catalytic sites, solubilization and activation of precipitated reactants, and increased mobility of reactants. As the a_w was increased from 0.42 to 0.67, the rate of methyl linoleate oxidation decreased (Tables 12 and 13). This decreased rate of methyl linoleate oxidation is attributed to dilution of reactants in the water associated with capillary hydration of the model system. Rockland (1974) reported that capillary hydration begins to occur in this model system as the a_w approaches 0.65. Labuza et al. (1972) reported similar observations of a decreased rate of methyl linoleate oxidation as capillary hydration occurred.

As the a_w of the model food system was decreased from 0.23 to 0.11, the rate of methyl linolenate oxidation increased. Labuza (1980 a) theorized that a decrease of moisture below the B.E.T. monomolecular moisture content increases the rate of lipid oxidation because many of the peroxides which would be deactivated by hydrogen bonding to water molecules remain activated because of an insufficient number of water molecules to bind to the peroxides. The increased oxidation rate of methyl linoleate at a_w below the B.E.T. moisture content may also be due to a change in the physical state of reactants, a gaseous-solid state reaction may predominate at a low a_w where water is limited.

Oxygen Effect

The rate of methyl linoleate oxidation was affected by the oxygen content in the storage container. The rate of methyl linoleate oxidation was greatest when the model food system was stored with limited headspace volume (Tables 12 and 13). Dugan (1976) reported that many volatile products are produced during oxidation of unsaturated lipids.

These volatile products may include reactive free radicals which react with methyl linoleate. These volatile reactive products would be more concentrated in the headspace of the TDT cans, which could catalyse the degradation of methyl linoleate in the model system stored in TDT cans. No attempt was made to confirm that volatile reactants were present in the headspace of the containers.

The degradation of methyl linoleate did not deviate from zero order kinetics when the model system was stored with a molar ratio of oxygen to methyl linoleate of 12:1 (303 cans) or 0.13:1 (TDT cans). Data in Figure 16 show that oxygen uptake by the model food system was limited when the molar ratio of oxygen to methyl linoleate was 1:1. Methyl linoleate would not be expected to be completely oxidized in the TDT can since the molar ratio of oxygen to methyl linoleate was $\leq$1:1 (i.e., 0.13:1).

Methyl linoleate degradation can occur in the absence of oxygen. Unsaturated lipid degradation occurs in three stages, initiation, propagation, and termination. Initiation is characterized by the direct attack of oxygen on a double bond of the unsaturated lipid to produce free radicals. Propagation is characterized by the reaction of the pre-formed radicals with unsaturated fatty acids in which hydrogen atoms are abstracted from the unsaturated fatty acids. This abstraction of hydrogen atoms can occur in the presence or absence of oxygen. In this experiment, sufficient oxygen was present in the two storage containers to allow initiation of lipid oxidation. After consumption of all of the oxygen, methyl linoleate degradation could occur by free radical reactions. The rate of degradation of methyl linoleate remained constant and thus, no deviation from the zero order kinetic plot was observed.

Effect of α-Tocopherol

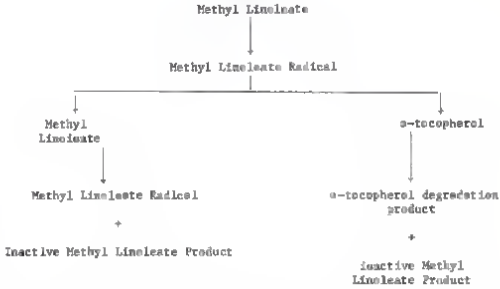
The rate of methyl linoleate degradation was affected by the initial concentration of α-tocopherol (Tables 12 and 13). The rate of methyl linoleate oxidation decreased as the concentration of α-tocopherol was increased. As discussed previously, the rate of α-tocopherol degradation increased as the initial concentration of α-tocopherol was increased.

Interaction with α-tocopherol

The previously discussed storage parameters (a_w, temperature, initial α-tocopherol concentration, and storage container oxygen content) affected the rate of degradation of methyl linoleate and α-tocopherol in a model system. The identity of the reactant(s) which controlled the zero order degradation rate of α-tocopherol or methyl linoleate was not determined in this experiment.

Previous research (Urano and Matsue, 1976, Gruger and Teppel, 1970 a and b) has demonstrated that free radicals produced from lipid autoxidation can react with either α-tocopherol or methyl linoleate. These free radicals formed from the oxidation of methyl linoleate during storage of the model food system are proposed to react with methyl linoleate or α-tocopherol in competitive reactions.

The relation between the rates of α-tocopherol and methyl linoleate degradation suggests that α-tocopherol and methyl linoleate are competing for a common reactant. This common reactant is probably a free radical formed by methyl linoleate oxidation. An example of one competitive reaction is:



The reaction of α-tocopherol with methyl linoleate free radicals would be favored by an increased concentration of α-tocopherol. An increased concentration of α-tocopherol would decrease the rate of methyl linoleate degradation in two ways. 1) lower the probability of methyl linoleate reaction with the free radicals, and 2) the rate of regenerating of free radicals formed as an end product of methyl linoleate oxidation would be decreased.

The reactions involving α-tocopherol and methyl linoleate are not as simple as depicted by the previously proposed mechanism. The complicating factors need to be discussed to indicate the inadequacy of this simple proposed mechanism. As discussed previously, oxygen is required for oxidation of α-tocopherol and initiation of methyl linoleate oxidation, but not for free radical/methyl linoleate reaction. Dugan (1976) reported that three different free radicals are produced during the induction period of methyl linoleate oxidation, and that the reactivity

of each radical varies. Scission of the methyl linolenate molecule, which may produce additional free radicals, may occur during oxidation. Gregor and Tappel (1970, a and b) reported that metal ions will catalyze α-tocopherol degradation in the presence of free radicals. They also reported that the free radicals formed from lipid oxidation are dependent upon the type of metal ion present and the concentration of the free radicals. The proposed mechanism for α-tocopherol and methyl linolenate degradation does not account for some of the possible components of methyl linolenate and α-tocopherol degradation. Rather, it is a simple explanation of the competing reactions between methyl linolenate and α-tocopherol occurring in the dehydrated model food system.

Activation Parameters

The apparent activation energies (Ea) for degradation of methyl linolenate in a dehydrated model food system containing α-tocopherol are shown in Table 14. The Ea's for methyl linolenate degradation ranged from 11.1 to 18.2 Kcal mol^{-1}. There was no significant difference in the Ea's ($p<0.01$). The Ea's were not consistently affected by a_w, initial α-tocopherol concentration, or oxygen content in the storage containers. These data indicate that the mechanism of degradation of methyl linolenate was not changed as a function of the storage parameters studied.

Oxygen Uptake

In addition to monitoring the stability of methyl linolenate and α-tocopherol in the model system containing unsaturated fat, the oxygen consumed by the model system during storage was monitored. The results of oxygen uptake studies provide estimates of both the amount of oxygen required for oxidation of the model system components and the rate of oxygen consumption by reactions occurring in the model system.

The patterns of oxygen uptake by the samples containing methyl linoleate and α-tocopherol are shown in Figure 16. Samples which did not contain methyl linoleate or α-tocopherol were used as controls. The control samples did not consume a detectable amount of oxygen during the storage period indicating that the consumption of oxygen in the model system is dependent on the presence of methyl linoleate or α-tocopherol. Quantitation of the amount of oxygen required for oxidation of α-tocopherol in the model system showed that these values were too small to measure, indicating that the oxygen uptake was dependent upon the methyl linoleate in the model system.

The rates of oxygen uptake and the calculated induction periods of the model system containing methyl linoleate and α-tocopherol are reported in Table 15. The rate of oxygen uptake was a function of the a_w of the samples. The slowest oxygen uptake occurred when the a_w was near the B.E.T. monomolecular moisture content. As the a_w was increased or decreased from 0.23, the rate of oxygen consumption increased. This effect of a_w on the rate of oxygen consumption was consistent with the previously discussed effect of a_w on the rate of methyl linoleate and α-tocopherol degradation during storage. These data indicate that the rate of oxygen uptake could be used as an indication of methyl linoleate stability.

The lag time before samples began to consume oxygen was also affected by the a_w. The induction periods, however, did not follow a discernible pattern with respect to the a_w or initial α-tocopherol concentration of the model system. These data indicate that the initial requirement for oxygen in the model system varies and was affected by uncontrolled factors.

One uncontrolled factor in these oxygen uptake studies was the concentration of oxygen bound by reactants in the model system before oxygen uptake was monitored. Camphell et al. (1974) reported that unsaturated lipids must be oxygenated before lipid oxidation could occur. These authors reported that an apparent anaerobic oxidation of unsaturated lipids observed during the oxygen uptake inductive period was due to the oxidation of lipids which had bound oxygen prior to initiation of the storage study. Oxygen uptake was observed only after the initial bound oxygen was depleted by lipid oxidation. Similar reactions may have occurred during storage of the model system containing methyl linoleate and α-tocopherol. In these experiments, the model system was equilibrated in a vacuum desiccator containing saturated salt solutions. The vacuum in the desiccators (650 mm Hg vacuum) may not have completely de-oxygenated the methyl linoleate since Camphell et al. (1974) reported that de-oxygenation of pure methyl linoleate required exposure to 1520 mm Hg vacuum. Thus, the reported length of the induction periods in this study may not be related to the rate of lipid oxidation, but rather to the amount of methyl linoleate which was oxygenated prior to measurement of oxygen uptake.

Biological Activity of α-Tocopherol After Storage

A rat bioassay was used to estimate the biological activity of α-tocopherol after storage in model systems containing no lipids, saturated lipids, or unsaturated lipids. Possible degradation products of α-tocopherol produced during storage of the model system were tentatively identified. These degradation products were synthesized and their biological activity on α-tocopherol was determined.

Following 48 days of vitamin E depletion, each rat was given 20 g of repletion diet daily for four days. To prevent major changes in the diet presented to the rats, the repletion diets consisted of a blend of model system and vitamin E deficient diet. The standard control model systems consisted of freshly prepared model system containing known concentrations of α-tocopherol. The concentration of α-tocopherol in the stored model food systems used in this experiment ranged from 11.6 to 61.7 μg α-tocopherol per g model system (Table 16). The stored model systems were blended with model system containing no added α-tocopherol so that the final concentration of dietary α-tocopherol would fall within the range of the standard control model systems. The repletion diets were prepared by blending model system fortified with α-tocopherol with the vitamin E deficient diet. All of the repletion diets contained the same ratio of model system to vitamin E deficient diet (3:7). Preparation of the repletion diets in this manner minimized variations in the response of the groups of rats due to a difference in the amount of model system in the diet.

The average diet consumption of the groups of rats ranged from 17.5 to 18.6 g diet per rat per day. No one group of rats consumed a significantly different amount of diet ($p \leq 0.05$). These data indicate that all groups of rats consumed the same amount of diet, and that the presence of stored model system or degradation products in the model system did not affect the dietary intake of the rats. The group average weights of the rats ranged from 320.75 to 350.11 g per rat. The mean weight of any group was not significantly different from the other ($p \leq 0.05$), indicating that consumption of the various diets did not cause a significant weight change during the 4 day repletion period. All of the rats

appeared healthy following the repletion period indicating that the various diets tested did not cause any overt responses in the rats.

The standard curves relating plasma α-tocopherol concentration and pyruvate kinase (PK) activity to standard α-tocopherol dietary levels are shown in Figures 17 and 18. The response of plasma α-tocopherol concentration and PK activity were related to dietary α-tocopherol levels as a curvilinear function. This relationship was maintained between the range of 0 to 2.5 mg α-tocopherol per Kg body weight per day for plasma α-tocopherol, and between 0.08 to 1.6 mg α-tocopherol per Kg body weight per day for plasma PK activity. Machlin et al. (1978) reported similar curvilinear responses for plasma α-tocopherol and PK activity between the ranges of 0.1 to 3.0 mg α-tocopheryl acetate per Kg body weight per day.

Machlin et al. (1978) reported that plasma aspartate aminotransferase (AspAT) activity responded to dietary α-tocopherol levels in a pattern similar to PK response. The AspAT activities in the rat plasma from the present experiment did not vary from sample to sample. The plasma AspAT activities were determined using a pre-mixed assay kit (Sigma Co.). The AspAT activity of plasma from α-tocopherol-depleted rats was approximately the same as the AspAT activity of plasma from the depleted rats. Thus, the plasma AspAT activity was not used as an indicator of the biological activity of α-tocopherol. Rather, plasma PK activity and plasma α-tocopherol concentrations were used as indicators of the α-tocopherol biological activity.

The biological vitamin E activities of the stored model food systems are reported in Table 16. The response ranges of plasma α-tocopherol concentrations or PK activities were too large to allow determina-

tion of the equivalent concentration of dietary α-tocopherol for each individual rat, as the group average responses, plus or minus one standard error, were used as a measure of dietary α-tocopherol equivalents. The concentrations of dietary α-tocopherol corresponding to the plasma α-tocopherol concentration (or PK activity), within one standard error of the group mean, are reported in Table 16.

The model food system containing no fat was stored until the concentration of α-tocopherol decreased from 195.5 to 61.7 μg per g as determined by the HPLC procedure (Table 16). The equivalent α-tocopherol concentration of the model food system as measured by the rat bioassay was 291.3 (plasma α-tocopherol bioassay) or 87.4 (PK activity bioassay) μg α-tocopherol per g model system. The bioassay utilizing plasma α-tocopherol concentration revealed a concentration of α-tocopherol in the model system much higher than was actually present. This relationship was not expected because α-tocopherol degradation products did not exhibit any significant effect on plasma α-tocopherol concentration. No theories are presented to explain this effect.

The PK activity bioassay of the same model system fed to the same group of rats indicated that the α-tocopherol remaining after storage was biologically active. The α-tocopherol equivalent of the range of the mean group PK activity plus or minus one standard error did include the concentration of α-tocopherol remaining in the model system. These data indicate that the α-tocopherol remaining in the model system after storage was biologically active, and α-tocopherol degradation products in the stored model system did not contribute measurable α-tocopherol activity.

A model system containing 0.01 g hydrogenated coconut fat and 132.2 g α-tocopherol per g model system was stored until the α-tocopherol concentration decreased to 36.7 µg per g, as measured by the HPLC procedure. The α-tocopherol equivalents of the range of the mean group PK activity and plasma α-tocopherol concentration plus or minus one standard error included the α-tocopherol concentration of the stored model system (Table 16). Thus, the biological activity of the remaining α-tocopherol is not affected by storage of a model food system containing 1% hydrogenated coconut fat.

A model system containing 1% methyl linolenate and 125.5 µg α-tocopherol per g model system was stored until α-tocopherol had degraded to 11.6 µg per g, as measured by the HPLC procedure (Table 16). The equivalent α-tocopherol ranges obtained from one standard error around the mean group responses of plasma α-tocopherol concentration and PK activity were both 0-9.6 µg α-tocopherol per g model system. Thus, the biological activity of α-tocopherol in a stored model system containing methyl linolenate is lower than the HPLC estimate of the concentration of α-tocopherol remaining in the model system.

The stored model system containing methyl linolenate did contain oxidizing unsaturated lipids. The maximum amount of oxidizing lipids in the mixed repletion diet was 3 mg per rat per day. This low level of oxidized lipid may have catalyzed oxidation of α-tocopherol during digestion or absorption. Further rat feeding studies would be required to identify the factor(s) which caused a decreased biological activity of α-tocopherol in the model system containing unsaturated lipids.

The biological activity of α-tocopheryl oxide, α-tocopheryl quinone, and α-tocopherol dimer, which are suspected degradation products formed

during storage of α-tocopherol in the model systems, are shown in Table 17. Dietary α-tocopheryl oxide did not cause an increase in plasma α-tocopherol, but did cause a change in the pyruvate kinase activity. This indicates that α-tocopheryl oxide was not converted to α-tocopherol during digestion, absorption, or transport in the blood, even though α-tocopheryl oxide did affect the PK activity. These data also indicate that α-tocopheryl oxide was transferred through the blood stream intact, but exhibited intracellular α-tocopherol activity. Boyer (1951) reported that α-tocopheryl oxide could be reduced to α-tocopherol in vitro by ascorbic acid or other reducing compounds indicating the biological activity of α-tocopheryl oxide may result from its intracellular reduction of α-tocopheryl oxide to α-tocopherol.

The biological activity of α-tocopheryl quinone is reported in Table 17. Like α-tocopheryl oxide, α-tocopheryl quinone caused no increase in plasma α-tocopherol concentration, but did cause a PK activity response. Alpha-tocopherol quinone would not be expected to be converted into α-tocopherol in a biological system (Hughes and Tove, 1980), but MacKenzie et al. (1950) reported that α-tocopheryl quinone was cure muscular dystrophy in animals, and Machlin (1980) reported that α-tocopheryl quinone caused a PK activity response in vitamin E depleted rats. These data indicate that the rate of muscle breakdown and associated PK activity were decreased by dietary α-tocopheryl quinone, as seen in the present experiment.

The biological activity of α-tocopheryl dimer is reported in Table 17. Alpha-tocopheryl dimer exhibited less than 10% biological α-tocopherol equivalence (wt/wt) by both the plasma α-tocopherol and PK activity bioassays. These data indicate that significant amounts of the dimer

were not converted into α-tocopherol and that the α-tocopheryl dimer was not responsible for any PK response in these experiments. Calaway and Draper (1963) reported that α-tocopheryl dider is the major α-tocopherol breakdown product in the rat and that this product is excreted from the rat. Since the dimer of α-tocopherol is a major α-tocopherol breakdown product, it would not be expected to exhibit any α-tocopherol activity, as was shown in this experiment.

The possible formation of α-tocopheryl oxide and α-tocopheryl quinone during storage of α-tocopherol in the model food systems has been previously discussed. Both of these compounds exhibited α-tocopherol activity by the PK bioassay, and the presence of either of these degradation products in the model food systems would be expected to elevate the PK bioassay estimate of α-tocopherol. Alpha-tocopheryl dimer did not exhibit a significant effect on the PK activity or plasma α-tocopherol concentration.

Alpha-tocopheryl oxide and α-tocopheryl quinone did not exhibit a significant effect on the plasma α-tocopherol concentration, indicating that the presence of these compounds would not affect the plasma α-tocopherol concentration estimate of dietary α-tocopherol. Quantitation of these compounds in the model food system was not done, therefore, their actual contribution to α-tocopherol biological activity in the stored model food system cannot be determined.

SUMMARY AND CONCLUSIONS

The storage stability of α-tocopherol in a dehydrated model food system at various water activities (a_w), storage container oxygen contents, storage temperatures, and lipid composition was investigated. The effect of these parameters on the biological activity of α-tocopherol following storage in a dehydrated model food system was also studied.

Alpha-tocopherol degradation in the model systems containing no lipid or fortified with a saturated lipid could be described by first order kinetics. The rate of α-tocopherol degradation in the model system containing no fat was most rapid at the highest a_w studied (0.65) and decreased as the a_w was lowered to 0.10. These data indicate that α-tocopherol degradation required a water soluble reactant and that the activity or availability of this reactant increased as the a_w was increased. The effect of a_w on the degradation rate of α-tocopherol was similar when the model system contained no lipids or saturated lipids.

Other storage parameters influenced the storage stability of α-tocopherol in a model food system containing no fat. The rate of α-tocopherol degradation was affected by the amount of oxygen present in the storage container. Alpha-tocopherol in a model system stored with a molar ratio of oxygen to α-tocopherol of 1450:1 degraded more rapidly than α-tocopherol in a model system stored with a 15:1 molar ratio of oxygen to α-tocopherol. The effect of headspace oxygen was most pronounced at higher storage temperatures and higher a_ws indicating the

effects of mass transport, solubility of oxygen, and the rate of diffusion of reactants through the model system influenced the degradation rate of α-tocopherol. The thermodynamic parameters including apparent activation energies and Gibb's free energy of activation were independent of a_w or storage container oxygen content, indicating that the mechanism of α-tocopherol degradation did not change due to variation of these storage parameters.

The loss of α-tocopherol during storage in a dehydrated model food system containing an unsaturated lipid, methyl linoleate, could be modeled by zero order kinetics. The loss of α-tocopherol did not follow simple zero order kinetics theory since the degradation rate of α-tocopherol was affected by the initial α-tocopherol concentration. The rates of α-tocopherol and methyl linoleate loss were affected by the ratio of α-tocopherol to methyl linoleate, indicating that methyl linoleate and α-tocopherol were degrading by a complex competing reaction.

Alpha-tocopherol was most stable at a a_w near the B.E.T. monomolecular value of 0.24. The rate of α-tocopherol degradation was increased as the a_w was raised to the multilayer region (0.42), or lowered below the B.E.T. value (a_w of 0.11). The rate of α-tocopherol loss decreased as the a_w was increased from the multilayer moisture region to the capillary hydration region (a_w of 0.67). The rate of α-tocopherol degradation was increased when the volume of headspace was limited indicating an increased concentration of reactive volatile decomposition products or a change in the composition or concentration of the reactants formed during storage of the model food system. Alpha-tocopherol degraded to a level of 20 μg per g in the model system stored with 0.05 mmol oxygen, while α-tocopherol in the model food system stored in containers having

4.8 nmol oxygen degraded to 0 μg per g model system. These results indicate that oxygen was required for degradation of α-tocopherol and that α-tocopherol degradation may be inhibited in the absence of oxygen.

Methyl linoleate in the model system degraded in a zero order fashion. The rate of methyl linoleate degradation was affected by the a_w in a pattern similar to the effect of a_w on the rate of α-tocopherol degradation. Methyl linoleate degraded more rapidly when stored in a container with limited headspace. This indicates that the concentration and/or composition of reactive compounds were influenced by storage of the model food system with limited headspace, and these differences caused variations in methyl linoleate degradation. The rate of methyl linoleate degradation decreased as the initial concentration of α-tocopherol was increased, indicating that methyl linoleate and α-tocopherol are interacting in a competitive reaction.

The α-tocopherol remaining after storage in a model food system containing no lipids or saturated lipids was biologically active, as determined by a rat bioassay. The α-tocopherol remaining in a stored model food system containing oxidizing methyl linoleate had a decreased biological activity, indicating that some of the α-tocopherol may be degraded during digestion or absorption in the presence of oxidizing lipids. Alpha-tocopheryl oxide and α-tocopheryl quinone exhibited α-tocopherol biological activity even though an increase in plasma α-tocopherol concentration did not occur. Alpha-tocopheryl dimer did not cause a marked increase of the plasma α-tocopherol concentration or biological response, indicating that this degradation product had little α-tocopherol activity.

REFERENCES

Abe, K., Yuguchi, Y. and Katsui, G. 1975. Quantitative determination of tocopherols by high-speed liquid chromatography. J. Nutr. Sci. Vitaminol. 21:183.

Ames, S. R. 1972. Occurrence in Foods. In "The Vitamins," 2nd ed. Academic Press, New York.

Bach, J. 1974. Thiamine stability in a dehydrated model food system during storage. M.S. thesis. Michigan State University, East Lansing, MI.

Barr, A., Goodnight, S. H., Sall, J. R., and Helwig, J. T. 1979. A User's Guide to SAS. Sparks Press, Raleigh, NC.

Bauernfeind, J. D. 1977. The tocopherol content of feed and influencing factors. Crit. Rev. Food Sci. Nutr. 8:337.

Bieri, J. G. and Evarts, R. P. 1973. Tocopherols and fatty acids in American diets. J. Am. Dietet. Assoc. 62:147.

Binder, H. J. and Spiro, H. M. 1967. Tocopherol deficiency in man. Am. J. Clin. Nutr. 20:594-601.

Boehringer Mannheim Co. 1973. Pyruvate kinase. In "Biochemica Information I," p. 154.

Booth, V. H. 1964. The α-tocopherol content of forage crops. J. Sci. Food Agric. 15:342.

Boyer, P. D. 1951. The preparation of a reversible oxidation product of α-tocopherol, α-tocopheroxide and of related oxides. J. Am. Chem. Soc. 73 733.

Bruhn, J. C. and Oliver, J. C. 1978. Effect of storage on tocopherol and carotene concentrations in alfalfa hay. J. Dairy Sci. 61:980.

Brunauer, S. 1945. "The Adsorption of Gases and Vapors." Princeton University Press, Princeton, NJ.

Bunnell, R. H., Keating, J., Quaresimo, A., and Furman, G. K. 1965. Alpha-tocopherol content of feeds. Am. J. Clin. Nutr. 17:1.

Bunyan, J., McHale, D., Green, J., and Marcinkiewicz, S. 1961. Biological potencies of ε-, ζ$_1$-tocopherols and 5-methyltocol. Br. J. Nutr. 15:253.

Casei, F. I., Mannswirth, J. W. and Nair, P. P. 1972. Biosynthesis of heme in vitamin E deficiency. Ann. N. Y. Acad. Sci. 203:93.

Cahell, C. A. and Ellis, N. R. 1942. The vitamin E content of certain varieties of wheat, corn, grasses and legumes as determined by rat bioassay. J. Nutr. 23:633.

Campbell, I. M., Caton, R. E. and Crozier, D. N. 1974. Complex formation and reversible oxygenation of free fatty acids. Lipids 9: 916-920.

Catignani, G. L., Chytil, F. and Darby, W. 1974. Vitamin E deficiency: Immunochemical evidence for increased accumulation of liver xanthine oxidase. Proc. Nat. Acad. Sci. 71:1969.

Cavins, J. F. and Inglett, G. E. 1974. High-resolution liquid chromatography of vitamin E isomers. Cereal Chem. 51:605.

Century, B. and Horwitt, M. K. 1965. Biological availability of various forms of vitamin E with respect to different indices of deficiency. Fed. Proc. 24 906.

Chau, H. E. and Labuza, T. P. 1974. Antioxidant effectiveness in intermediate moisture content model systems. J. Food Sci. 39:479.

Cort, W. M., Mergean, W. and Greese, A. 1978. Stability of alpha- and gamma-tocopherol: Fe^{3+} and Cu^{2+} interactions. J. Food Sci. 43:797.

Cmallany, A. S. and Draper, H. H. 1963. Dimerization of α-tocopherol in vitro. Arch. Biochem. Biophys. 100:335.

Davis, A. W. and Moore, T. 1941. Interaction of vitamins A and E. Nature 147:794.

Diplock, A. T. 1974. Possible stabilizing effect of vitamin E on microsomal, membrane-bound, selenide-containing proteins and drug-metabolizing enzyme systems. Am. J. Clin. Nutr. 27 995.

Dugan, L. 1976. Lipids. In "Principles of Food Science, Part 1, Food Chemistry." Marcel Dekker, Inc., New York.

Evans, H. M., Emerson, O. H. and Emerson, G. A. 1936. The isolation from wheat germ oil of an alcohol, α-tocopherol, having the properties of vitamin E. J. Biol. Chem. 113:319.

Evans, H. E. and Bishop, K. S. 1922. On the existence of a hitherto unrecognized dietary factor essential for reproduction. Science 56:650.

Eyring, H. 1935. Activated complex and the absolute rate of reactions. J. Chem. Phys. 3:107.

Fahrenholtz, S. R., Doleiden, F. H., Trozzolo, A. M., and Lamola, A. A. 1974. On the quenching of singlet oxygen by α-tocopherol. Photochem. and Photobio. 20:505.

Fennema, O. R. 1976. Water and ice. In "Principles of Food Science. Part I., Food Chemistry," ed. O. R. Fennema, Marcel Dekker, Inc., New York.

Ferholz, F. 1938. On the constitution of α-tocopherol. J. Am. Chem. Soc. 60:700.

Foote, C. S., Ching, T. and Geller, G. G. 1974. Chemistry of singlet oxygen - XVIII. Rate of reaction and quenching of α-tocopherol and singlet oxygen. Phytochem. and Phytobio. 20:511.

Frampton, V. L., Skinner, W. A., Cambour, P., and Bailey, F. S. 1960. α-Tocopurple, an oxidation product of α-tocopherol. J. Am. Chem. Soc. 82:4632.

Fraser, A. C., Hickman, J. R., Simmons, N. G., and Sherrett, M. 1956. Studies on the effects of treatment with chlorine dioxide on the properties of wheat flour. IV. - The biological properties of untreated, normally treated and overtreated flours. J. Sci. Food Agric. 7:464.

Frazer, A. C. and Lines, J. G. 1967. Studies on changes in flour tocopherols following aging and the treatment of the flour with chlorine dioxide. J. Sci. Food Agric. 18:203.

Gramm, G. W., Ellessin, C. W. and Inglett, G. E. 1970. Distribution of tocopherols within the corn kernel. J. Am. Oil Chem. Soc. 47:337.

Green, J. 1958. The distribution of tocopherols during the life-cycle of some plants. J. Sci. Food Agric. 9:801.

Green, J. and Sooyau, J. 1969. Vitamin E and the biological antioxidant theory. Nutr. Abst. Rev. 39:321.

Gruger, E. H. and Tappel, A. L. 1970a. Reactions of biological antioxidants: I. Fe(III)-catalyzed reactions of lipid hydroperoxides with α-tocopherol. Lipids 5:326.

Gruger, E. H. and Tappel, A. L. 1970b. Reactions of biological antioxidants: II. Fe(III)-catalyzed reactions of methyl linoleate hydroperoxides with derivatives of coenzymes Q and vitamin E. Lipids 5:332.

Gutfinger, T. and Letan, A. 1974. Quantitative changes in some unsaponifiable components of soya bean oil due to refining. J. Sci. Food Agric. 25:1143.

Halton, P. and Fisher, E. A. 1937. Studies on the storage of wheaten flour. II. The absorption of oxygen by flour stored under various conditions. Cereal Chem. 14:267.

Heidelbaugh, N. D., Yeh, C. F. and Karel, M. 1971. Effects of model system composition on autoxidation of methyl linolenate. Agric. Food Chem. 19:140.

Hertin, D. C. and Drury, E. E. 1969. Alpha-tocopherol content of cereal grains and processed cereals. J. Agric. Food Chem. 17:785.

Hughes, P. E. and Tove, S. B. 1980. Synthesis of α-tocopherolquinone by the rat and its reduction by mitochondria. J. Biol. Chem. 255(15):7095.

Jensen, A. 1969. Tocopherol content of seaweed and seaweed meal. III. - Influence of processing and storage on the content of tocopherols, carotenoids and ascorbic acid in seaweed meal. J. Sci. Food Agric. 20:622.

Kebay, M. E. and Gilbert, J. J. 1977. A new rotifer-based assay for tocopherol. Lipids 12:875.

Kanner, J., Harel, S. and Mendel, E. 1979. Content and stability of α-tocopherol in fresh and dehydrated pepper fruits (Capsicum annuum L.). J. Agric. Food Chem. 27:1316.

Karrer, P. and Fritsche, H. 1938. Die niedrigere homologen des α-tocopherols. Helv. Chim. Acta. 21:1234 [In Bauernfeind, J. C. 1977. The tocopherol content of food and influencing factors. Crit. Rev. Food Sci. Nutr. 8:337].

Karrer, P., Escher, H., Fritzsche, H., Keller, E., Ringier, B., and Solomon, H. 1938. Konstitution und bestimmung des α-tocopherols und einiger ähnlicher verbindungen. Helv. Chim. Acta. 21:939 [In Bauernfeind, J. C. 1977. The tocopherol content of food and influencing factors. Crit. Rev. Food Sci. Nutr. 8:337).

Ke, P. J. and Ackman, R. G. 1973. Bunsen coefficient for oxygen in marine oils at various temperatures determined by an exponential dilution method with a polarographic oxygen electrode. J. Am. Oil Chem. Soc. 50:429.

Kirk, J. R. 1978. Influence of water activity on stability of vitamins in dehydrated foods. Presented at Second International Symposium on Properties of Water in Relation to Food Quality and Stability, Osaka, Japan, Sept. 10-16.

Knapp, F. W. and Tappel, A. L. 1961. Comparison of the radiosensitivities of the fat-soluble vitamins by gamma irradiation. J. Agric. Food Chem. 9:430.

Kodicek, E., Braude, R., Kon, S., and Mitchell, K. 1959. The availability to pigs of nicotinic acid in tortilla baked from fresh maize treated with lime-water. Br. J. Nutr. 13:363.

Kofler, M., Sommer, F. F., Halliger, H. E., Schmidli, H., and Vecchi, M. 1972. Physicochemical properties and assay of the tocopherols. Vitamin. Hormon. 30:407.

Lahuis, T. F. 1968. Serpine phenomenia in foods. Food Tech. 22:263.

Labuza, T. P. 1972. Nutrient losses during drying and storage of dehydrated foods. CRC Crit. Rev. Food Tech. 2:217.

Labuza, T. P. 1976. Interpretation of sorption data in relation to the state of constituent water. In "Water Relations of Foods," ed. R. B. Duckworth, Academic Press, New York.

Labuza, T. P. 1980a. The effect of water activity on reaction kinetics in food deterioration. Food Tech. 34(4):36.

Labuza, T. P. 1980b. Enthalpy/entropy compensation in food reactions. Food Tech. 34(2):67.

Labuza, T. P., Kaldelbaugh, N. D., Silver, M., and Karel, M. 1972. Oxidation at intermediate moisture contents. J. Am. Oil Chem. Soc. 48:86.

Labuza, T. P., McNally, L., Gallagher, D., Hawkes, J., and Hurtado, F. 1972. Stability of intermediate moisture foods. I. Lipid oxidation. J. Food Sci. 37:154.

Labuza, T. P., Tsuyuki, H. and Karel, M. 1969. Kinetics of linoleate oxidation in model systems. J. Am. Oil Chem. Soc. 46:409.

Laidler, K. J. 1963. "Chemical Kinetics," 2nd ed. McGraw-Hill Co., New York.

Lange, W. 1950. Cholesterol, phytosterol, and tocopherol content of food products and animal tissues. J. Am. Oil Chem. Soc. 27:414.

Laffler, J. E. 1955. The interpretation of enthalpy and entropy data. J. Org. Chem. 31:533.

Lawicki, P. P., Bunk, G. C., Peterson, P. L., and Labuza, T. P. 1978. Determination of factors controlling accurate measurement of A_w by the vapor pressure manometric technique. J. Food Sci. 43:244.

Litov, R. E., Dillard, C. J., Downey, J., Irving, D., Sagel, M., Gee, D., and Tappel, A. L. 1978. Measurement of in vivo lipid peroxidation via pentane: Effect of vitamin E, selenium and polyunsaturated fats and assay for acute toxic reactions. Fed. Proc. 37:706.

Livingston, A. L., Nelson, J. W., and Kohler, G. D. 1968. Stability of α-tocopherol during alfalfa dehydration and storage. J. Agr. Food Chem. 16:492.

Lasdon, E. D., Hauswirth, J. W., Kudig, F.D., and Emir, P. P. 1972. Dependence of heme biosynthesis on vitamin E status and age modulation by phenobarbitol. Fed. Proc. 31:713.

Machlin, L. J. 1980. Unpublished data. Hoffmann-LaRoche, Inc., Nutley, New Jersey.

Machlin, L. J., Gabriel, E., Spiegel, H. E., Horn, L. E., Brin, M., and Nelson, J. 1978. Plasma activity of pyruvate kinase and glutamic oxalacetic transaminase as indices of myopathy in the vitamin E deficient rat. J. Nutr. 108:1963.

MacKenzie, J. B., Kneeskrantz, B., Ulick, S., and Milhorat, A. T. 1950. The biological activity of α-tocopheryl hydroquinone and α-tocopherylquinone. J. Biol. Chem. 183:655.

Maisuny, J. P., Labuza, T. P., Wallace, D. E., and Karel, M. 1966. Autoxidation of methyl linoleate in freeze-dried model systems. I. Effect of water on the autocatalyzed oxidation. J. Food Sci. 31:878.

Martinez, P. and Labuza, T. P. 1968. Rate of deterioration of freeze-dried salmon as a function of reintive humidity. J. Food Sci. 33:241.

Mattill, H. A. 1927. The relation of vitamins A and E to fertility in the male rat. Am. J. Physiol. 79:305.

Matill, H. A., Carman, J. S. and Clayton, M. N. 1924. The nutritive properties of milk. III. The effectiveness of the X substrate in preventing sterility in rats on milk rations high in fat. J. Biol. Chem. 61:729.

McHale, D., Mamalis, P., Green, J., and Marcinkiewicz, S. 1958. Tocopherols. Part I. Synthesis of 7-methyltocol. J. Chem. Soc. 37:1600.

McLaughlin, P. J. and Weihrauch, J. L. 1979. Vitamin E content of foods. J. Am. Dietet. Assoc. 79:647.

Mohr, D. H. 1980. Oxygen mass transfer effects on the degradation of vitamin C in foods. J. Food Sci. 45:1432.

Moore, T., Sharman, I. M. and Ward, H. J. 1957. The destruction of vitamin E in flour by chlorine dioxide. J. Sci. Food Agric. 8:97.

National Academy of Sciences. 1980. "Recommended Dietary Allowances." 9th ed. National Academy of Sciences, Washington, D.C.

Olcott, H. and Emerson, O. H. 1937. Antioxidants and the autoxidation of fats. IX. The antioxidant properties of the tocopherols. J. Am. Chem. Soc. 59:1008.

Privett, O. S. and Blank, M. L. 1962. The initial stages of autoxidation. J. Am. Chem. Soc. 39:465.

Pudelkiewicz, W. J. and Matterson, L. D. 1960. A fat-soluble material in alfalfa that reduces the biological availability of tocopherol. J. Nutr. 71:143.

Quast, D. G. and Karel, M. 1972. Effects of environmental factors on the oxidation of potato chips. J. Food Sci. 37:584.

Ramanujan, K. A. and Anantakrishnan, C. P. 1958. Tocopherol stability in milk. Indian J. Dairy Sci. 11:179.

Rao, M. K. and Perkins, E. G. 1972. Identification and estimation of tocopherols and tocotrienols in vegetable oils using gas chromatography — mass spectrometry. J. Agr. Food Chem. 20:240.

Robeson, C. D. 1943. Crystalline natural α- and γ-tocopherol. J. Am. Chem. Soc. 65:1660.

Rockland, R. B. 1960. Saturated salt solutions for static control of relative humidity between 5° and 40°C. Anal. Chem. 32:1375.

Rockland, L. B. 1969. Water activity and storage stability. Food Tech. 23:1241.

Rothe, M., Feldheim, W. and Thomas, B. 1958. _____. Ernaehrungsforschung 3:386 [In Bauernfeind, J. C. 1977. The tocopherol content of food and influencing factors. Crit. Rev. Food Sci. Nutr. 8:337].

Salwin, H. 1959. Defining minimum moisture contents for dehydrated foods. Food Tech. 13:594.

Salwin, H. 1963. Moisture levels required for stability in dehydrated foods. Food Tech. 17:1114.

Sheikh, B., Huang, H. S. and Zielinski, W. L. 1977. High performance liquid chromatographic analysis of supplemental vitamin E in feed. JAOAC 60:137.

Smith, L. I., Irwin, W. B. and Ungnade, H. E. 1939. Isolation of tocopherol red — a product from tocopherol. J. Am. Chem. Soc. 61:2424.

Soderhjelm, P. and Andersson, B. 1978. Simultaneous determination of vitamins A and E in foods and feeds by reversed phase high-pressure liquid chromatography. J. Sci. Fd. Agric. 29:697.

Stevens, B., Small, R. D. and Perez, S. B. 1974. The photoperoxidation of unsaturated organic molecules — XIII. O_2'Δg quenching by α-tocopherol. Photochem. and Photobio. 20:515.

Sure, B. 1924. Dietary requirements for reproduction. II. The existence of a specific vitamin for reproduction. J. Biol. Chem. 58:693.

Thompson, J. S., Fox, J. B. and Landmann, W. A. 1962. The effect of water and temperature on the deterioration of freeze-dried beef during storage. Food Tech. 16:131.

Touchstone, J. C. and Dobbins, M. F. 1978. "Practice of thin layer chromatography," Wiley-Interscience Publ., New York.

Tsen, C. C. 1961. An improved spectrophotometric method for the determination of tocopherols using 4,7-Diphenyl-1,10 phenanthroline. Anal. Chem. 33:849.

Tunny, J., Rimmergardt, L. C. and Helmer, R. L. 1969. Effect of oxygen uptake on quality of cooked, freeze-dried combination of foods. J. Agr. Food Chem. 17:1360.

Urano, S. and Matsuo, M. 1976. A radical scavenging reaction of α-tocopherol with methyl radical. Lipids 11:380.

Van Niekerk, F. J. 1973. The direct determination of free tocopherols in plant oils by liquid-solid chromatography. Anal. Biochem. 52:533.

Voth, O. L. and Miller, R. C. 1958. Interactions of tocopherol with proteins and amino acids. Arch. Biochem. Biophys. 77 191.

Waters, R. D., Kesterson, J. W. and Braddock, R. J. 1976. Method for determining the α-tocopherol content of citrus essential oils. J. Food Sci. 41:370.

Widicus, W. A. and Kirk, J. R. 1979. High pressure liquid chromatographic determination of vitamins A and E in cereal products. JAOAC 62:637.

Widicus, W. A. and Kirk, J. R. 1980. Unpublished data. University of Florida, Gainesville, Florida.

Williams, R. C., Schmidt, J. A. and Emery, R. A. 1972. Quantitative analysis of fat soluble vitamins by high pressure liquid chromatography. J. Chrom. Sci. 10:494.

Wilson, F. W., Kodicek, E. and Booth, V. H. 1962. Separation of tocopherols by gas-liquid chromatography. Biochem. J. 84:524.

Young, L. G., Lun, A., Poe, J., Forshew, R. P., and Edmeades, D. 1975. Vitamin E stability in corn and mixed feed. J. Anim. Sci. 40:495.

BIOGRAPHICAL SKETCH

Warren A. Widicus was born on March 16, 1954, in East St. Louis, Illinois. After graduating from Triad High School in St. Jacob, Illinois, he attended the University of Illinois — Champaign-Urbana, receiving a Bachelor of Science degree in food science in May, 1976. He began his master's program in food science and human nutrition in September of 1976 at Michigan State University. After electing to bypass his master's degree, he transferred to the University of Florida in September, 1978, and plans to graduate in December, 1980. He plans to begin his career at the Quaker Oats Company in Barrington, Illinois.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

James R. Kirk, Chairman
Professor of Food Science and Human Nutrition

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Lynn B. Bailey
Assistant Professor of Food Science and Human Nutrition

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

John A. Carnell
Professor of Statistics

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Jesse P. Gregory III
Assistant Professor of Food
Science and Human Nutrition

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

H. Ansen Moye
Professor of Food Science
and Human Nutrition

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December 1980

Jack L. Fry
Dean, College of Agriculture

Dean, Graduate School